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MULIBREY NANISM

Characterization of Hypogonadism, Infertility and Tumors

Susann Karlberg

ACADEMIC DISSERTATION

To be publicly discussed, with the permission of the Faculty of Medicine,
University of Helsinki, in the Niilo Hallman Auditorium,
Children's Hospital, on June 15th 2012,
at 12 noon

Helsinki 2012

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ISBN 978-952-10-8032-6 (paperback)
ISBN 978-952-10-8033-3 (PDF)
<http://ethesis.helsinki.fi>
Unigrafia Oy
Helsinki 2012

To my beloved family

"Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature by the careful investigation of cases of rare forms of disease".

William Harvey 1657

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ABSTRACT

Background and aims: Mulibrey nanism (MUL; OMIM 253250) is an autosomal recessive disorder belonging to the Finnish disease heritage and currently classified as a peroxisomal disorder. MUL is characterized by intrauterine-onset growth restriction, typical dysmorphic features, restrictive perimyocardial heart disease, and severe insulin resistance. The causative gene, *TRIM37*, is located on chromosome 17q22-q23 and encodes for a peroxisomal protein (TRIM37) with ubiquitin E3-ligase activity, suggesting its role in proteasomal protein degradation. All 19 mutations identified to date, including 4 in Finnish patients, are likely to produce a non-functioning protein.

For many decades, the clinical care and follow-up of Finnish MUL patients were based at the Children's Hospital in Helsinki, generating a unique clinical experience and data set for this patient group. In follow-up, it became evident that a substantial proportion of these patients developed hypogonadism, were infertile, and had an increased risk of developing tumors. Thus, the principal focus of this study was to characterize the hypogonadism and infertility associated with this disorder, and furthermore to define the tumors and tumor predisposition in MUL, both clinically and immunohistochemically.

Patients and methods: A total of 92 Finnish patients (0.7-77 years) were included in the study; 22 post pubertal females participated in the female hypogonadism study, and 28 male patients participated in the male hypogonadism study. All hospital records were evaluated, and physical, laboratory, and radiological examinations were performed according to clinical protocols. Biopsies (taken on clinical grounds) and autopsy samples were used for histological and immunohistochemical studies. In addition, 15 freshly frozen samples from sporadic ovarian fibrothecomas were analyzed for the role of TRIM37 in the development of these tumors.

Results: All MUL patients developed hypogonadism due to a primary gonadal defect and were either infertile or severely subfertile. Women demonstrated spontaneous puberty, incomplete breast development, and early irregularity of menstrual periods with subsequent ovarian failure. Their ovaries were hypoplastic and their uteri were small. The men also experienced spontaneous puberty with somewhat small testes characterized by varying degrees of degeneration and few mature germ cells. Semen samples showed either severe oligoasthenozoospermia or azoospermia. No spontaneous pregnancies had been conceived among the MUL patients. Four men had undergone infertility treatment, which in one case was successful, resulting in the delivery of a healthy child.

The MUL patients displayed a high frequency of both benign and malignant tumors especially of endocrine origin. A total of 232 tumorous lesions in several different organs were discovered in the patient cohort. Histologically, the architecture of several organs was disturbed, indicating aberrant organogenesis. One of the most frequent tumors was ovarian fibrothecomas, which are fairly uncommon in the general population, found in 55% of the female patients.

Conclusions: The study of inherited monogenic disorders has contributed substantially to our understanding of gene function and human disease. In MUL, mutations in *TRIM37* lead to failure of sexual maturation in both females and males, and fertility is seriously compromised. In addition, the patients are at a very high risk for developing benign and malignant tumors in several different organs. This study suggests a role for TRIM37 protein in the cellular functions governing gametogenesis, gonadal function, and proliferation.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to by their roman numerals I-IV. In addition, some previously unpublished results are presented.

- I Karlberg S, Tiitinen A, Lipsanen-Nyman M. Failure of Sexual Maturation in Mulibrey Nanism. *N Engl J Med* 2004;351:2559-2560.
- II Karlberg S, Toppari J, Karlberg N, Nurmio M, Karikoski R, Jalanko H, Lipsanen-Nyman M. Testicular failure and male infertility in the monogenic Mulibrey nanism disorder. *J Clin Endocrinol Metab* 2011;96:3399-3407.
- III Karlberg N, Karlberg S, Karikoski R, Mikkola S, Lipsanen-Nyman M, Jalanko H. High frequency of tumours in Mulibrey nanism. *J Pathol* 2009;218:163-171.
- IV Karlberg S, Lipsanen-Nyman M, Lassus H, Kallijärvi J, Lehesjoki AE, Bützow R. Gynecological tumors in Mulibrey nanism and role for RING finger protein TRIM37 in the pathogenesis of ovarian fibrothecomas. *Modern Pathol* 2009;22:570-578.

Publication III has previously appeared in the thesis of Niklas Karlberg (2009). The original publications are reproduced with the kind permission of their respective copyright holders.

ABBREVIATIONS

AMH	anti-Müllerian hormone
CV	coefficient of variation
E1	ubiquitin-activating enzyme
E2	ubiquitin-conjugating enzyme
E3	ubiquitin ligase
FSH	follicle-stimulating hormone
GH	growth hormone
GnRH	gonadotropin-releasing hormone
HIF-1 α	hypoxia-inducible factor-1 α
HPG	hypothalamic-pituitary-gonadal
HRT	hormone replacement therapy
ICSI	intracytoplasmic sperm injection
i.e.	id est (that is)
IR	insulin resistance
IUGR	intrauterine growth restriction
IVF	<i>in vitro</i> fertilization
KS	Klinefelter syndrome
LH	luteinizing hormone
LOH	loss of heterozygosity
MRI	magnetic resonance imaging
MUL	Mulibrey nanism
POF	premature ovarian failure
pVHL	von Hippel-Lindau protein
PBD	peroxisomal biogenesis disorder
RBCC	RING-B-Box-Coiled-Coil
SGA	small for gestational age
SRS	Silver-Russell syndrome
TESE	testicular sperm extraction
TRIM	tripartite motif
TS	Turner syndrome
UPS	ubiquitin-proteasome system
US	ultrasound
VEGF	vascular endothelial growth factor
VHL	von Hippel-Lindau disease
WHO	World Health Organization

1. INTRODUCTION

A rare disease is classified as such when it affects a limited number of individuals (<1:2000 in Europe and <1:1250 in the USA) (Ong and Devuyst 2011). Therefore, by this definition, more than 5000 rare diseases exist (Schieppati et al. 2008), and the number of affected patients could number 30 million in Europe and 25 million in the USA (Ong and Devuyst 2011). Rare diseases are mostly genetic disorders, which are often chronic, disabling, and affecting life expectancy. Thus, they may be a severe burden for the affected patients and families. Lately, it has been increasingly recognized that rare diseases are an important medical and social issue (Schieppati et al. 2008). The study of inherited monogenic disorders has contributed greatly to our understanding of gene function and the pathophysiology of diseases, but there is also increasing evidence that monogenic defects play a significant, and previously underestimated, role in complex disorders (Ropers 2010).

The Finnish disease heritage comprises 36 rare monogenic diseases that are overrepresented in Finland (Norio 1973, 2003). These diseases have been the subject of intensive genetic and molecular research over the past 20 years, and to date, the genetic background of all the disorders has been characterized (Kestilä et al. 2010). These studies have often provided novel information on biological processes and metabolic pathways essential for normal cell function and development (Peltonen et al. 1999).

Mulibrey nanism (MUL; OMIM 253250) is a dysmorphic growth disorder belonging to the Finnish disease heritage and first described by Perheentupa and colleagues in the early 1970s (Perheentupa et al. 1970, 1973). The name is an acronym from muscle hypotonicity (MUscle), hepatomegaly (Liver), enlarged ventricles of the central nervous system (BRain), and yellowish dots of the ocular fundi (EYe). The term nanism, adapted from Latin, stands for short stature. For many decades, the clinical care and follow-up of MUL patients in Finland were based at the Children's Hospital in Helsinki, generating a unique clinical experience and data set for this patient group, which constitute the foundation of this study.

2. REVIEW OF THE LITERATURE

2.1 The Hypothalamic-Pituitary-Gonadal Axis

The adequate function of the hypothalamic-pituitary-gonadal (HPG) axis is essential for normal gonadal development, sex steroid production, and fertility. Defects at any level of the axis can lead to hypogonadism and subsequent impairment of fertility. The HPG axis is fully active during fetal life (Kaplan et al. 1976) and during the newborn period (Winter et al. 1975), but thereafter becomes relatively quiescent until puberty (Terasawa and Fernandez 2001).

Reactivation of the HPG axis, manifested by an increased synthesis and pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, indicates the onset of puberty. GnRH stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland, which in turn stimulate the ovaries and testes to produce the sex steroids estrogen and testosterone, respectively. FSH and LH are both produced by the same cells — the gonadotropes — in the pituitary, in proportions that are at least in part defined by the GnRH pulse frequency (Krsmanovic et al. 2009).

The sex steroids, estrogen and testosterone, exert a negative feedback inhibition on both hypothalamic GnRH and pituitary gonadotropin release, together with inhibin B and follistatin (Figure 1). Inhibin B is a gonadal glycoprotein, which specifically regulates FSH secretion (Illingworth et al. 1996). The peptide follistatin is produced in many tissues and exerts its negative feedback effects by inhibiting activin, a stimulatory peptide. LH and FSH induce the final stages of gonadal development, leading to the production of mature gametes and sex steroids (Grumbach 2002).

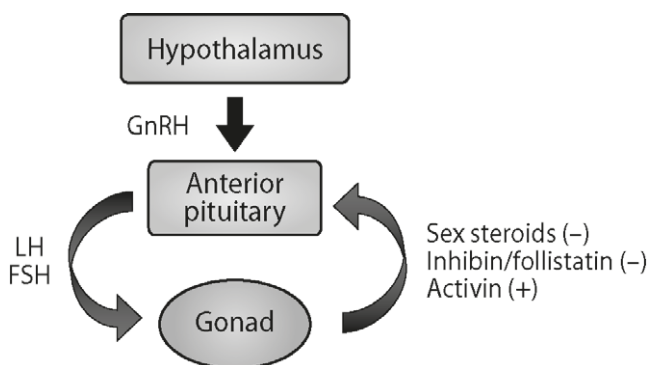


Figure 1. The HPG axis. GnRH, gonadotropin-releasing hormone, LH, luteinizing hormone, FSH, follicle-stimulating hormone. Modified from Achermann and Jameson 1999.

2.1.1 HPG Axis in Infancy and Childhood

Hypothalamic GnRH secretion begins by the end of the first trimester (Kaplan et al. 1976). At birth, FSH and LH levels are low because of an inhibitory effect of placental steroids, especially estrogen (Grumbach 2005). Within 2 weeks of age, when the placenta-derived steroids have dispersed, a reactivation of the HPG axis occurs. This “minipuberty” results in an increase of FSH and LH and subsequently gonadal sex steroid levels (Schmidt and Schwarz 2000). During this period, gonadotropin levels reach their maximum between 4 and 10 weeks. In males, LH levels predominate, whereas in females, FSH levels predominate.

This period of postnatal pituitary activation is considered to be important for normal reproductive development in boys, since during this period, there is an increase in the number of Sertoli and germ cells, growth of the penis and testes, and augmented testicular testosterone secretion (Grumbach 2005, Lewis and Lee 2009, Kuiri-Hänninen et al. 2011a). The importance of the minipuberty for female reproductive development is not equally well understood (Kuiri-Hänninen et al. 2011b). After the transient activation of the pituitary, gonadotropin levels gradually decline (Isidori et al. 2008). Already in childhood, the gonads secrete small amounts of estrogen and testosterone (Mitamura et al. 1999, 2000), but only a sufficient increase in their plasma levels will induce the development of the secondary sex characteristics and the pubertal growth spurt.

2.1.2 Puberty

Puberty is the transitional period from childhood to adulthood, during which the secondary sex characteristics appear, the growth spurt occurs, marked physiological changes take place, and fertility is achieved. Both genetic and environmental factors regulate the timing of puberty. The mechanisms that initiate puberty are not completely understood, but involve genes such as *KISS1* (encoding for the neuropeptide family of kisspeptins), and a shift in the balance of inhibitory neurotransmitters, such as GABA (gamma-aminobutyric acid), and neurostimulators, such as glutamate (Plant 2008, Belchetz et al. 2010). The end point of the pubertal process is the ability to reproduce.

The development of the secondary sex characteristics is driven by both the increase in GnRH stimulated sex steroids and adrenal androgens. Adrenarche, which develops independently of HPG-axis maturation, occurs about two years before the onset of puberty. Approximately two years before the first physical signs of puberty, there is also an increase in the nocturnal pulsatile release of GnRH, leading to an increased secretion of LH at night. In addition to LH, girls also show an increased secretion of FSH (Manasco et al. 1997). The nocturnal LH pulses gradually increase, both in amplitude and frequency, as the onset of puberty advances, and finally also occur during the daytime. In males, testos-

terone levels increase quickly and a circadian rhythm is established where the testosterone levels are markedly higher in the early morning than during the rest of the day. In girls, there is not an equally clear circadian rhythm in estradiol levels, but they tend to peak during the morning as well, and increase with Tanner stage (Norjavaara et al. 1996).

Generally, pubertal development proceeds in a specific order, described by Tanner stages 1 through 5 for breast, genital and pubic hair growth (Marshall and Tanner 1969, 1970). In girls, the appearance of breast tissue (Tanner breast stage 2, B2) is usually the first sign of puberty (Marshall and Tanner 1969). In boys, the first sign is usually the enlargement of the testes to a volume of 3 ml or more (Tanner genital stage 2, G2) (Marshall and Tanner 1970, Biro et al. 1995). The mean age for achieving B2 and G2 in Finnish children is 10.8 and 12.2 years, respectively (Ojajärvi 1982). Menarche, i.e. the first menstrual bleeding, is quite a late occurrence in female puberty, manifesting approximately 2.3 years after reaching B2. It may, however, sometimes be the first sign of puberty (Marshall and Tanner 1969, Tanner and Whitehouse 1976). Mean age at menarche for Finnish girls is 12.9 years (Niinikoski et al. 2007).

Normal growth can be divided into intrauterine, infant, childhood, and pubertal growth, finally resulting in adult height (Karlberg et al. 1987a,b, Karlberg 1989, Clayton and Gill 2001). Pubertal growth is characterized by an increased tempo (the pubertal growth spurt), which usually in boys occurs at Tanner G3. The gradually increasing secretion of the gonadal sex steroids mostly drives the pubertal growth spurt. In addition, rising growth hormone (GH) levels also contribute. In fact, GH is compulsory for optimal gonadal growth and development of secondary sex characteristics (Bordini and Rosenfield 2011). In selective GH resistance (Laron syndrome), patients possess small testes and a micropenis, poor breast and sexual hair development, and lack of pubertal growth spurt (Laron 2002). In girls, the growth spurt is often the first sign of puberty, together with B2 (Tanner and Whitehouse 1976). The highest velocity of the pubertal growth spurt is usually reached at Tanner stage 3 in girls, and stage 4 in boys. After this, growth velocity gradually decreases, during which time menarche usually occurs in girls (Tanner and Whitehouse 1976).

2.2 Ovarian Health

Healthy ovarian function is essential for the general health of a woman and for the production of sex steroids, which are needed for the development of the genital tract and for bone density (De Vos et al. 2010). In the embryo, primordial germ cells migrate from the yolk sac to the urogenital ridge, which in the female embryo becomes the ovary. The oogonia proliferate to ultimately form a total of 7×10^6 oocytes in the ovaries by about 20 weeks of gestation. After this, there is a continued loss of ovarian follicles through programmed cell death, apoptosis (Baker 1963, Hsueh et al. 1994, 1996, Vaskivuo et al. 2001). At

the time of birth, each ovary has a fixed number of primordial follicles, usually about 1×10^6 . This number is constantly reduced throughout life because of atresia and recruitment to ovulation (Gosden and Faddy 1998) (Figure 2). The newborn infant has already lost over 80% of her original germ cell pool, and by puberty, the number has decreased to 300,000. Less than 500 of the original 7×10^6 (0.007%) oocytes are ovulated during the entire reproductive life span of a woman (Anasti 1998).

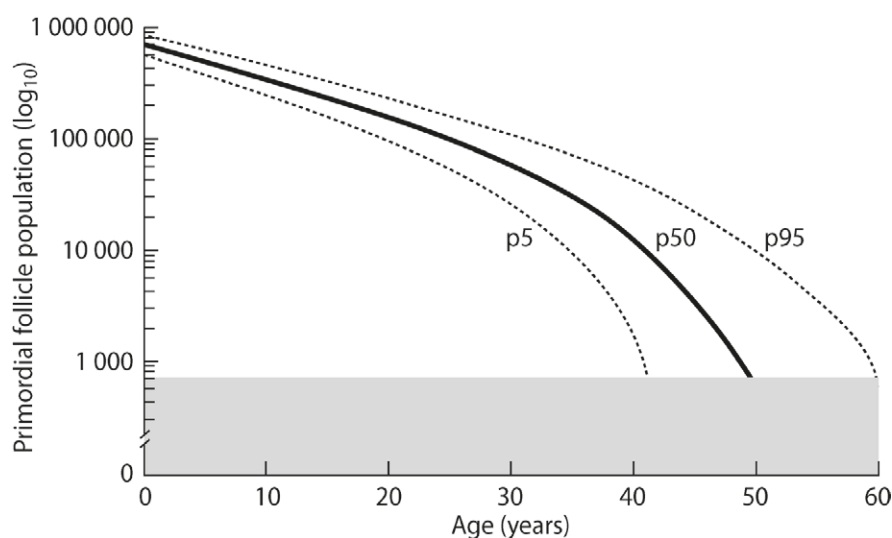


Figure 2. Decline of the ovarian follicular reserve. Shaded area indicates level of follicle population at which ovarian failure or menopause occurs. p5=5th percentile, p50=median, p95=95th percentile. Modified from De Vos et al. 2010.

2.2.1 Normal HPG Axis during Adult Female Life

During adult female life, an active pulsatile GnRH stimulated secretion of FSH and LH is imperative for the maintenance of secondary sex characteristics and for sexual function (Krsmanovic et al. 2009) (Figure 3). The development of a cyclic, regular ovulatory menstrual cycle results from stringently controlled interactions between the hypothalamus, pituitary, ovaries, and genital tract. The normal menstrual cycle can be divided into two main phases: the follicular (proliferative) phase and the luteal (secretory) phase. The follicular phase begins on the first day of the cycle, which is also the first day of menstruation. This phase is characterized by the growth and maturation of the dominant follicle and by proliferation of the uterine endometrium. Low basal levels of LH stimulate the theca cells to produce androgens. While theca cells lack aromatase, which is needed for conversion of androgens to estrogens, the androgens are transported to the adjacent granulosa cells, where FSH stimulates their conversion to estrogens. The primordial follicles are composed of an oocyte sur-

rounded by a single layer of granulosa cells. Under the stimulation of FSH, the primordial follicles will grow into the preovulatory follicle stage, where many layers of granulosa cells and an outer layer of theca cells surround the oocytes. During the midfollicular stage, usually one dominant follicle will continue to grow while the other follicles that have been developing undergo atresia and degenerate (Hawkins and Matzuk 2008).

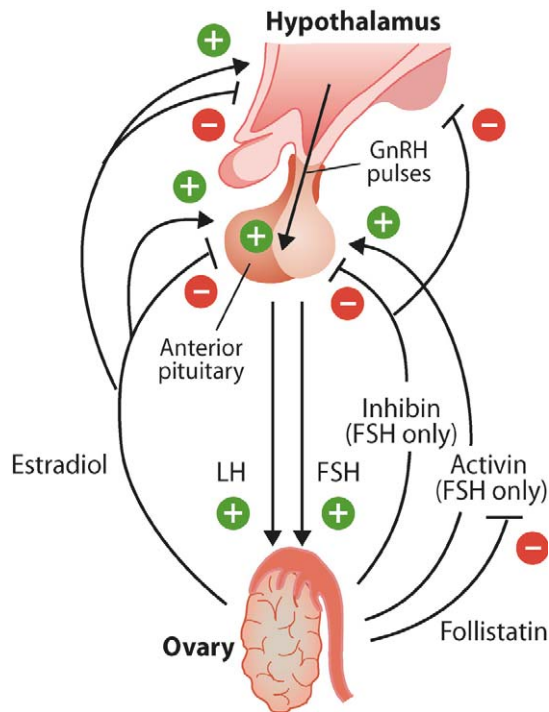


Figure 3. HPG axis in females. Adapted from Bellin et al. 2009.

FSH also stimulates the synthesis of inhibin B and activin by the follicles. Inhibin B provides negative feedback to selectively inhibit FSH synthesis and release, whereas activin amplifies LH action on theca cells. The resulting increasing inhibin B and estradiol levels consequently inhibit the secretion of FSH at the pituitary level. The rising levels of estradiol during the late follicular phase stimulate the release of LH. This surge in LH induces ovulation and the subsequent formation of the corpus luteum, which is formed from the theca and granulosa cells that remain from the dominant follicle after the release of the oocyte.

Ovulation indicates the beginning of the luteal phase, where the corpus luteum synthesizes progesterone and estrogen for two weeks to prepare the uterine endometrium for implantation of the fertilized egg. In case there is no implantation, the corpus luteum regresses, estrogen and progesterone levels fall, the endometrium will be discarded as menstrual bleeding, and a new

menstrual cycle will begin. The median length of a normal menstrual cycle is 28 days (range 25-30 days) (Treloar et al. 1967, Vollman 1977).

2.2.2 Premature Ovarian Failure

Many factors can lead to disturbed ovarian function, such as hypothalamic-pituitary disorders, adrenal dysfunction, or polycystic ovary syndrome; however, premature or primary ovarian failure implies that the primary defect lies in the ovary. Through the years, different terms have been used to describe deviations in healthy ovarian function, for example premature menopause, premature ovarian failure, and primary ovarian insufficiency. Recently, some authors have suggested that premature ovarian insufficiency (POI) would be a more accurate term than premature ovarian failure, since ovarian insufficiency can be used to describe a wider range of impaired ovarian function and POF can have a long and variable clinical course (Welt 2008, Nelson 2009, De Vos et al. 2010). However, no international consensus has thus far been reached (Shelling 2010), and in the literature, both terms continue to be used in parallel.

POF affects 1-2% of women under the age of 40 years and 0.1% of women younger than 30 years of age (Coulam et al. 1986), and is a major cause of female infertility. In Western populations, the median age for menopause is approximately 51 years (Morabia and Costanza 1998). POF refers to the development of amenorrhea due to depletion of ovarian follicles before the age of 40 years. The condition is characterized by amenorrhea (for at least 4 months), elevated FSH-levels (detected on at least two occasions one month apart), and sex steroid deficiency (Welt 2008). Women with POF present with primary or secondary amenorrhea, infertility, elevated gonadotropins, and low estrogen levels.

2.2.3 Pathophysiology of POF

Perception of the causes for POF requires an understanding of the highly organized and complex process of ovarian follicular maturation, folliculogenesis (Figure 4). Folliculogenesis implicates the continuous maturation of small primordial follicles to large ovulatory follicles. The follicle consists of the oocyte surrounded by supporting somatic cells (granulosa and thecal cells), which are essential for the growth and development of the follicle. The final outcome of folliculogenesis is either ovulation or follicular atresia. Usually, only one oocyte is released from the surface of the ovary in ovulation. The granulosa and thecal cells, which are regulated by the gonadotropins FSH and LH, synthesize and secrete hormones and growth factors. Many of the molecules that are important for the regulation of follicular maturation are also known to be defective in some cases of POF.

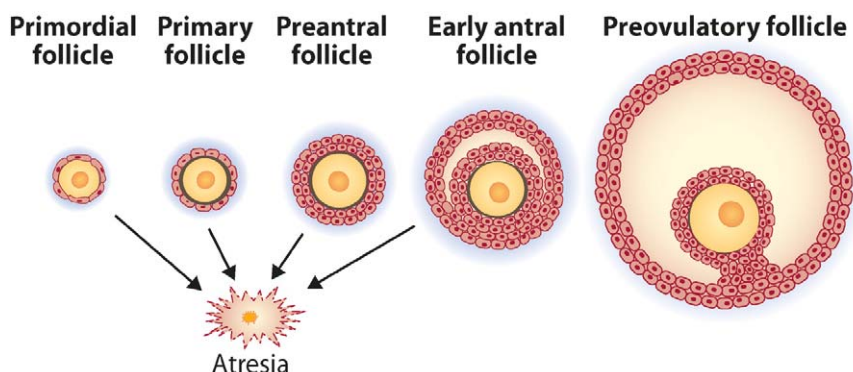


Figure 4. The process of folliculogenesis. Modified from Matzuk et al. 2002, Murphy 2010, Persani et al. 2010.

Generally, POF arises either from depletion or dysfunction of ovarian follicles (Rebar and Connolly 1990). Depletion of ovarian follicles can result from either a decreased number of oocytes formed during embryonic development or an increased rate of oocyte atresia during reproductive life. Follicular atresia occurs by means of programmed cell death, apoptosis. Thus, alteration in the rate of apoptosis may result in early ovarian failure. Follicle dysfunction, which is more uncommon, implies that follicles remain in the ovary, but a pathologic process prevents their normal function (Nelson 2009). To date, the causes of POF are substantially unknown (Woad et al. 2006). Identified causes include iatrogenic agents, such as chemotherapy or radiation therapy, autoimmune conditions, X-chromosome abnormalities, and autosomal genetic conditions. POF has a strong genetic component, but most causes of POF remain unknown and the genes that have been found explain only a modest portion of POF cases (Persani et al. 2010, Shelling 2010) (Figure 5).

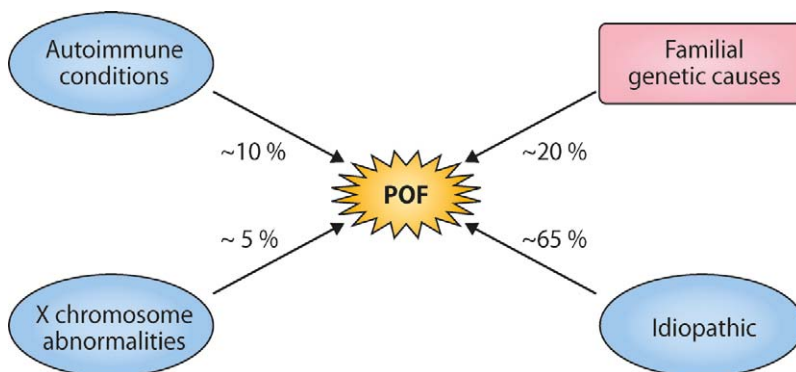


Figure 5. Causes of POF. Adapted from Shelling 2010.

2.2.4 Genetic Causes of POF and Female Infertility

Given that part of the phenotype of POF is infertility, it is understandable that reproductive disorders like POF do not have large family histories (Shelling 2010). Thus, they are difficult to study using traditional genetic methods such as linkage analysis. The genetic background of POF can be divided to syndromic forms (when the defect is a part of a complex phenotype involving other organs and tissues), and to non-syndromic forms (when ovarian failure develops apparently isolated in a woman before 40 years of age) (Persani et al. 2010).

2.2.4.1 Syndromic POF

X chromosome abnormalities, both structural and numerical, account for a significant number of the genetic causes of POF (Goswami and Conway 2005). Turner syndrome (TS) is the most common congenital cause of POF. TS occurs in approximately 1 in 2500 live female births, and is characterized by short stature, ovarian dysgenesis and neurocognitive problems. More than 50% of TS patients have the 45,X karyotype, whereas others demonstrate 46,XX with the paired X chromosome carrying some type of abnormality (Sybert and McCauley 2004). The ovaries of a 45,X fetus appear completely normal until 14-18 weeks gestation, after which there is loss of oocytes and fibrosis of the ovarian stroma (Weiss 1971, Singh and Carr 1966). As a result, the majority of TS girls have complete, or near-complete, follicular atresia already prenatally or during the first months or years of postnatal life. Up to 30% of TS girls may, however, experience some pubertal development and spontaneous pregnancies have been reported in 2-5% (Pasquino et al. 1997, Hovatta 1999, Hreinnsson et al. 2002). Women with mosaic TS (45,X/46,XX) may have menarche and a normal menstruation for many years (Sybert and McCauley 2004). Genetic imprinting, karyotype, and degree of mosaicism influence the variation in phenotype (Skuse et al. 1997, De Vos et al. 2010).

APECED (autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy; OMIM 240300) is an autosomal recessive disease belonging to the Finnish disease heritage. Patients present with two out of three major clinical symptoms, including Addison's disease, hypoparathyroidism, and chronic mucocutaneous candidiasis. Frequently, patients also exhibit various other autoimmune manifestations, such as thyroiditis, diabetes mellitus, and ovarian failure (Perheentupa 1996, 2006, Peterson and Peltonen 2005). The autoimmune regulator (*AIRE*) gene has been identified as the locus underlying the susceptibility to APECED (Finnish-German APECED Consortium 1997, Nagamine et al. 1997). To date, more than 60 variants of this gene have been found, and recent studies have also identified influences by other genetic loci, for example the human leukocyte antigen (HLA) complex (Mathis and Benoist 2007). In a Finnish study of 72 patients, hypogonadism was present in 60% of female patients over the age of 12 years (Perheentupa 1996). Male patients may also present with hypogonadism, but not as frequently as females (Perheentupa 2006).

Ovarian failure also results from mutations in *FOXL2* (forkhead box L2), leading to blepharophimosis-ptosis-epicanthus-inversus syndrome (BPES; OMIM 110100). BPES is an autosomal dominant condition characterized by complex eyelid malformation. Two forms of the disorder have been recognized: type I, in which affected females have POF, and type II, which is not connected with POF (Zlotogora et al. 1983). The *FOXL2* gene on chromosome 3q22-q23, encoding for a winged helix/forkhead transcription factor, is mutated in both BPES I and II (Crisponi et al. 2001). *FOXL2* plays an important role in ovarian differentiation and maintenance (Cocquet et al. 2002) and was the first human autosomal gene in which dominant mutations were implicated in ovarian dysfunction. *FOXL2* is mainly expressed by undifferentiated granulosa cells in the ovary (Pisarska et al. 2004). In *Foxl2* knockout mouse ovaries, a default granulosa cell differentiation leads to premature activation of primordial follicles and subsequent follicular depletion and atresia (Schmidt et al. 2004). Although mutations in *FOXL2* have been demonstrated in a few isolated cases of non-syndromic POF (Harris et al. 2002), other studies of phenotypically normal women with POF indicate that mutations in the *FOXL2* coding region are seldom associated with non-syndromic POF (De Baere et al. 2001, 2002, Bodega et al. 2004).

Ataxia telangiectasia (AT; OMIM 208900) is an autosomal recessive neurodegenerative disorder caused by mutations in the *ATM* (ataxia telangiectasia mutated) gene encoding for a protein kinase that is involved in cell cycle regulation and DNA repair (Shiloh 2003, Kastan and Bartek 2004). The disorder is characterized by uncoordinated movements, ocular telangiectasias, chromosome instability, radiosensitivity, immunodeficiency, and predisposition to cancer (Boder 1985, Savitsky et al. 1995, Lavin 2008). Female AT patients frequently present with ovarian failure and infertility due to gonadal hypoplasia with complete absence of mature oocytes (Miller and Chatten 1967, Persani et al. 2010). *Atm*-deficient mutant female mice are also infertile and have extremely small ovaries with lack of primordial and mature follicles and oocytes (Barlow et al. 1996). Nonetheless, no studies have been made into the potential role for the *ATM* gene in non-syndromic POF cohorts.

Classic galactosemia (OMIM 230400) is a rare, autosomal recessive disorder of galactose metabolism, with an estimated incidence of 1:23000-1:44000 in Western Europe (Bosch 2006). It is caused by deficiency of the GALT (galactose 1-phosphate uridylyltransferase) enzyme (Leslie et al. 1992), leading to the accumulation of galactose and its metabolites, which subsequently leads to abnormalities in glycosylation. Classic galactosemia causes a toxic neonatal syndrome that can be reversed with a lifelong galactose restricted diet; however, despite prompt dietary intervention, most patients already develop long-term complications at a young age, including delayed growth and speech, ataxia, tremor, and diminished bone mineral density (Waggoner et al. 1990, Bosch 2006). Female patients with classic galactosemia commonly demonstrate de-

layed pubertal development and amenorrhea, or oligomenorrhea. It was previously estimated that 60-70% of female patients develop POF, but follow-up of patients has revealed that the correct number is close to 100% (Kaufman et al. 1981, 1986, Waggoner et al. 1990, Gubbels et al. 2008). Nonetheless, pregnancies have been reported (Gubbels et al. 2008, Rubio-Gozalbo et al. 2010). Thus far, there is no evidence for an association between mutations in *GALT* and idiopathic POF (Mlinar et al. 2005). Limited data are available on the gonadal status of male patients with galactosemia, but fertility does not seem to be impaired (Rubio-Gozalbo et al. 2010).

2.2.4.2 Non-Syndromic POF

Despite rigorous efforts, no single gene on the X chromosome has been consistently found to be involved in the pathogenesis of POF to date. The fragile-X mental retardation 1 gene, *FMR1*, lies on Xq27 and is responsible for the development of fragile X syndrome (Jacquemont et al. 2007). Interestingly, POF has been linked to the untranslated region of the *FMR1* transcript (Sullivan et al. 2005). Expansion of a CGG repeat sequence in the 5' untranslated region of *FMR1* is associated with changes in the amount of *FMR1* mRNA and protein, and leads to varying phenotypes (Jacquemont et al. 2007). Expansions of over 200 repeats are considered to be a full mutation and they lead to the fragile X syndrome in males (Garber et al. 2006). Smaller expansions, between 55 and 200 repeats, are known as pre-mutations, which are unstable and may expand to full mutations when transmitted maternally (Maddalena et al. 2001). Women with pre-mutations have a ten times higher risk of developing POF, while those with a full mutation or a normal range of repeats are not at an increased risk of POF (Conway et al. 1998, Allen et al. 2007, Persani et al. 2009).

Bone morphogenetic protein 15 (BMP15) is a member of the TGF β superfamily and is specifically expressed in the oocyte during early folliculogenesis. This growth and differentiation factor is critical for follicular development as it regulates many processes in the granulosa cells (Dube et al. 1998, Shimasaki et al. 2004). The *BMP15* gene is located at Xp11.22 (Dube et al. 1998). Mutations in *BMP15* are associated with both primary and secondary amenorrhea in many worldwide POF cohorts with a prevalence of 1.5-12% (Persani et al. 2010). However, some studies have also failed to find an association between *BMP15* mutations and POF (Takebayashi et al. 2000, Chand et al. 2006). Thus the role of *BMP15* mutations in the etiology of POF remains uncertain, but they could potentially function as susceptibility factors for this condition.

Mutations in many autosomal genes have also been associated with POF. It would be reasonable to consider that the hormones associated with the HPG axis would constitute good candidate genes for regulating oocyte atresia. Abnormalities in the key regulating hormones, such as FSH, LH, and their receptors, are not frequent causes of POF, however. A recessive mutation in

the FSH-receptor gene (*FSHR*), mapping to 2p21, has been linked to POF in a number of Finnish families (Aittomäki et al. 1995). Other populations, however, have not commonly reported this mutation (Shelling 2010). All females with the *FSHR* mutation are infertile. Similarly, female *Fshr* knockout mice (FORKO) are also infertile, which is due to a block in folliculogenesis at a primary stage. Interestingly, an intra-ovarian injection of adenovirus expressing human *FSHR* gene in these mice is able to restore the responsiveness to FSH, and to re-establish ovarian folliculogenesis and continue the production of estrogen (Ghadami et al. 2010). Males homozygous for the *FSHR* mutation demonstrate varying degrees of spermatogenic failure but are not infertile by definition, indicating that normal FSH action is not as crucial for spermatogenesis as it is for ovarian follicle maturation (Tapanainen et al. 1997).

NR5A1 (nuclear receptor subfamily 5, group A, member 1), also known as SF1 (steroidogenic factor 1), is an orphan nuclear receptor, which is essential for gonadal development and ovarian steroidogenesis (Luo et al. 1994, Lin and Achermann 2008). Recently, the *NR5A1* gene, located at 11q13, has been shown to have a key role in ovarian development and function (Lourenço et al. 2009). The NR5A1 protein regulates the expression of central genes in the HPG axis that are involved in sexual development and reproduction, including *STAR* (encoding for steroidogenic acute regulatory protein), *AMH* (encoding for anti-Müllerian hormone) and *INHA* (encoding for inhibin alpha subunit). Newborn *Nr5a1*^{-/-} mice lack both gonads and adrenal glands, and exhibit diminished expression of gonadotropins (Luo et al. 1994), thus supporting a key role for *Nr5a1* in ovarian development and function in the mouse. Mutations of human *NR5A1* have been known to cause 46,XY disorders of sex development (Achermann et al. 1999), and were recently shown to cause POF in otherwise healthy 46,XX females (Lourenço et al. 2009).

The *FOXO3a* (forkhead box O3A) gene belongs, like *FOXL2*, to the forkhead gene family and encodes for a transcriptional regulator. The gene maps to 6Q21 and has a role in ovarian development and function (Watkins et al. 2006). *Foxo3a* knockout mice are sterile because of an accelerated activation of ovarian follicles. Once activated, follicles have a limited lifespan, meaning that in *Foxo3a* knockout mice, premature follicle activation induces an early death of most oocytes (Castrillon et al. 2003). To date, the relevance of this mutation for the development of POF in humans is still unknown. Despite the fact that one study observed eight variations in *FOXO3a* in women with POF (Watkins et al. 2006), the implications of these variations remain unclear (Cordts et al. 2011).

Growth differentiation factor 9 (GDF9) is a member of the TGFβ superfamily. The *GDF9* gene is located on chromosome 5q23 and is highly homologous to *BMP15*. GDF9 is specifically expressed in the oocytes and acts in a synergistic manner with BMP15 on folliculogenesis and granulosa cells (Yan et al. 2001). Mutations in *GDF9* have been found in non-syndromic POF patients (Otsuka et

al. 2011), but the results have not been consistent. Interestingly, *Gdf9*^{-/-} mice are infertile (Dong et al. 1996), while *Bmp15*^{-/-} mice are subfertile (Yan et al. 2001).

The *NOBOX* (*Newborn Ovary Homeobox*) gene encodes for an oocyte-specific transcription factor important in the transition from primordial to primary follicles (Rajkovic et al. 2001, Suzumori et al. 2002). While *Nobox*^{-/-} mice are infertile (Rajkovic et al. 2004), results in POF populations have been inconclusive; however, *NOBOX* mutations were recently found with a prevalence of 6.2% in one POF population (Bouilly et al. 2011), supporting a potential role for *NOBOX* mutations in the etiology of POF. Interestingly, *GDF9* seems to be directly regulated by *NOBOX*.

The inhibins are heterodimeric glycoproteins that are primarily produced in the gonads (Tong et al. 2003). They are members of the TGF β superfamily and consist of α and β subunits, which act at separate times of the menstrual cycle (Groome et al. 1996). The β subunit exists in two forms, A and B. Subsequently, there are two forms of inhibin: inhibin A (α - β A) and inhibin B (α - β B). Inhibin B is primarily a product of the developing follicle, whereas inhibin A is derived from the dominant follicle and the corpus luteum (Tong et al. 2003). Inhibin has been a strong candidate gene for POF because of its important role in regulating ovarian function both as a negative feedback regulator of FSH secretion and as a local paracrine factor in the ovary. A decline in inhibin levels is associated with a decline in the follicular reserve when approaching menopause. This decline of inhibin results in elevated FSH concentrations, increased follicular recruitment, and thus an acceleration in the rate of follicular depletion (Richardson et al. 1987, MacNaughton et al. 1992, Chand et al. 2010) (Figure 6). Thus, a mutation in the inhibin genes would result in a rise in the FSH concentration and follicle loss, leading to POF. Inhibin α is encoded by the *INHA* gene on chromosome 2q33 and it has been implicated in the etiology of POF. One mutation of the *INHA* gene, G769A, has been associated with non-syndromic POF with a prevalence of approximately 5%, depending on the population studied (Shelling et al. 2000, Shelling 2010, Marozzi et al. 2002, Dixit et al. 2004). However, while there are also asymptomatic carriers of the mutation, there is heterogeneity in the phenotypes of the *INHA* G769A mutation carriers (Chand et al. 2010), suggesting that this mutation is more of a susceptibility factor for POF (Shelling 2010). Interestingly, *Inha* knockout mice present with raised FSH levels, infertility, and sex cord-stromal tumors at an early age, showing that in mice inhibin B functions as a tumor suppressor in the gonads (Matzuk et al. 1992).

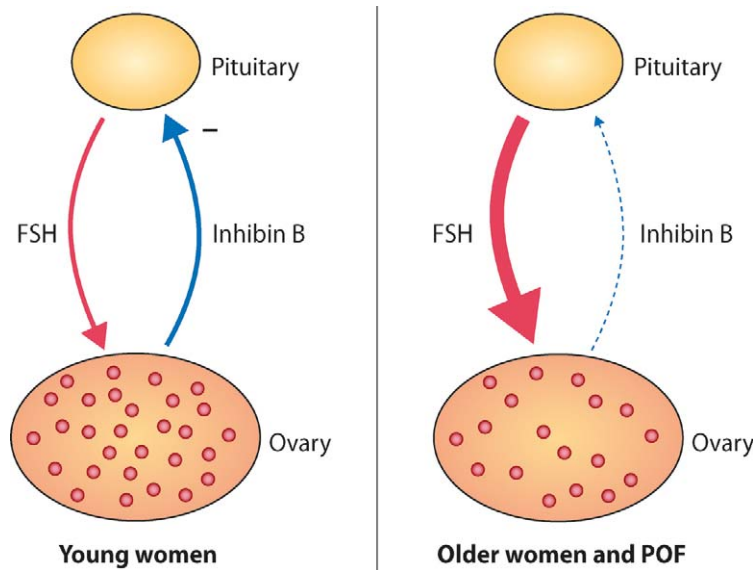


Figure 6. In older women and POF, a decreased number of follicles leads to a reduced production of inhibin B and subsequently increased FSH, in comparison to young women. Adapted from Shelling 2010.

2.2.5 Symptoms and Diagnosis of POF

POF can present as primary amenorrhea, i.e. absence of menses in a 15-year-old girl, or as secondary amenorrhea, i.e. the cessation of menses for 4 months or more. The patient may also have menopausal symptoms, such as hot flushes, night sweats, fatigue, mood changes, and sexual dysfunction. A prolonged exposure to reduced estrogen levels increases the risk for developing osteoporosis. Secondary amenorrhea with concomitantly raised FSH concentrations is suggestive of ovarian insufficiency (Goswami and Conway 2005).

2.2.6 Prognosis of POF

Treatment of POF comprises dealing with the menopausal symptoms, reducing the risk of osteoporosis, and dealing with the loss of fertility (Shelling 2010). Ovarian failure usually takes several years to develop, and occasionally ovarian function can intermittently be recovered in the early stages of the disorder, which may lead to ovulation and successful pregnancies in up to 5-10% of patients with ovarian insufficiency (Nelson et al. 1994, van Kasteren et al. 1999, Bidet et al. 2008). However, the likelihood for recovery of ovulation is not possible to predict. Accordingly, the only reliable fertility treatment is *in vitro* fertilization (IVF) with the use of ovum donation. In most cases of POF, the condition develops over time, such that there is a period of increasing FSH before

POF is established. During this time, cryopreservation of oocytes or ovarian tissue for later *in vitro* growth and maturation may be possible (Picton et al. 2008). While there are few symptoms of an approaching POF, early detection and identification of specific molecular defects would also provide a better opportunity for an early intervention.

2.3 Testicular Health

2.3.1 Normal HPG Axis during Adult Male Life

The adult testis has two major functions: the production of spermatozoa (fertility) and the secretion of testosterone (virility) (McLachlan et al. 2002a). In post-pubertal males, the GnRH pulse generator maintains the gonadotropin levels required for normal gonadal function (Isidori et al. 2008). FSH and LH stimulate steroid secretion and germ cell production in the testis (Figure 7). Male reproductive capacity is usually attained between the ages of 16 and 19 years. By the end of puberty, plasma testosterone concentration has reached adult male levels of 10-35 nmol/l, steady sperm production levels have been attained, and plasma concentrations of FSH and LH are in the adult range. The testis consists of the seminiferous tubules, comprising 80-85% of the testicular mass, surrounded by interstitial tissue containing the Leydig cells as well as lymphatic and blood vessels. LH stimulates the Leydig cells to produce testosterone, which partly is aromatized into estradiol. In turn, both testosterone and estradiol exert a negative feedback effect on LH and GnRH. Testosterone acts through androgen receptors (ARs), which are located on Sertoli cells, Leydig cells, and the peritubular myoid cells, but not on germ cells (Bremner et al. 1994, Johnston et al. 2001). FSH stimulates Sertoli cells to produce inhibin B, which has a negative feedback effect on the pituitary secretion of FSH.

The Sertoli cells lie on the basement membrane of the seminiferous tubules and extend all the way to the lumen. These cells provide nutrients, growth factors and support to the germ cells, and since each Sertoli cell only can support a given number of germ cells, they are the critical determinant of testicular size, seminiferous tubule development, and sperm count. Subsequently, in adults, inhibin B is a clinically applicable marker of Sertoli cell function, i.e. the gonadal reserve (Isidori et al. 2008).

In contrast to women, who experience a rather complete cessation of ovarian function in menopause, men have continuous spermatogenesis throughout their lifespan. During senescence, however, the feedback system of the HPG axis is altered, leading to a disorganized GnRH pulsatility and an increase in LH and FSH levels due to a diminished testicular responsiveness (Atwood et al. 2005, Vadakkadath et al. 2005), ultimately leading to a gradual decrease in male sexual function.

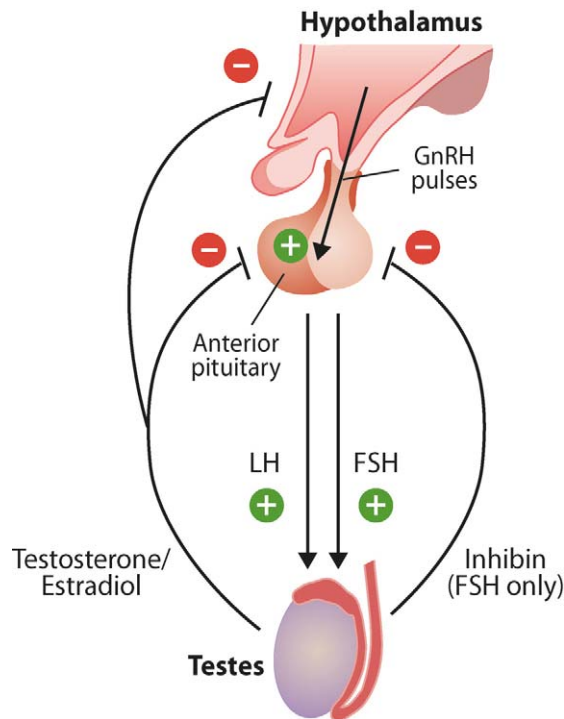


Figure 7. The HPG axis in males. Adapted from Bellin et al. 2009.

2.3.2 Testicular Failure and Infertility

The etiology of defective sperm production or function can be categorized as factors affecting the pre-testicular, testicular, or post-testicular level (Krausz 2011). Pre-testicular causes of infertility include two principal types of pathological conditions: hypogonadotropic hypogonadism (HH, also called secondary hypogonadism, both congenital and acquired) and coital disorders (such as erectile dysfunction and retrograde ejaculation). Post-testicular causes comprise all obstructive lesions of the seminal tract, infections and inflammations of the accessory glands, and autoimmune infertility. Testicular causes lead to primary testicular failure, or hypergonadotropic hypogonadism (also called primary hypogonadism), which accounts for up to 75% of male factor infertility (McLachlan and O'Bryan 2010, Krausz 2011). This condition is characterized by high serum FSH and LH and low testosterone levels. Adult men with primary testicular failure are infertile or subfertile.

2.3.3 Pathophysiology of Primary Testicular Failure

Primary testicular failure may result from a large number of different pathologies, including bilateral cryptorchidism, orchitis, testicular injury, iatrogenic causes, some systemic diseases, and genetic factors (Krausz 2011). Spermatogenesis is a complex process by which mature germ cells are produced through stringently controlled cell proliferation, meiosis, and differentiation. More than 2300 genes are estimated to be involved in this process (Schultz et al. 2003). Thus, it is very likely that mutations or polymorphisms in candidate genes involved in different stages of spermatogenesis are responsible for the majority of idiopathic forms of primary testicular failure.

During fetal life, primordial germ cells (PGC) differentiate into gonocytes, which give rise to spermatogonia postnatally. The spermatogonia are located close to the basement membrane of the seminiferous tubule, and they proliferate through mitosis to give rise to a reserve of undifferentiated stem cells, or more seldom, primary spermatocytes. The primary spermatocytes proceed via the first meiotic division to become secondary spermatocytes, and follow through a second meiotic division to become haploid round spermatids. The round spermatids finally undergo a series of molecular and morphological changes to produce mature spermatozoa (Figure 8). Subsequently, mature spermatozoa are released into the tubular lumen and advance to the epididymis for capacitation, the biochemical process by which sperm gains its mobility and the capability to fertilize an egg (Grootegeed et al. 2000).

Spermatozoa are produced continuously during the life of adult males. LH and FSH control the spermatogenic process by paracrine signaling; in the testis, LH receptors are found only in the Leydig cells, and FSH receptors are found only in the Sertoli cells. In response to LH, Leydig cells produce testosterone, which diffuses into the seminiferous tubules. Both FSH and testosterone are imperative for normal spermatogenesis; FSH is required for qualitative and quantitative normal spermatogenesis, and a high tubular testosterone concentration is crucial for germ cell maturation.

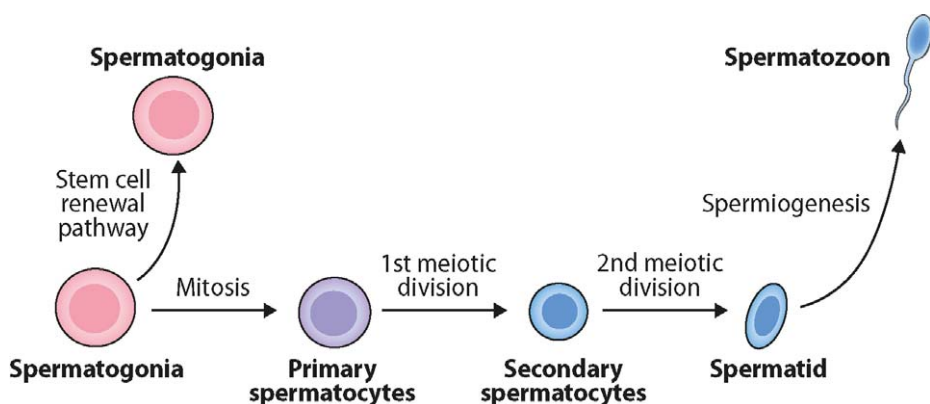


Figure 8. The process of spermatogenesis. Most spermatogonia proliferate to establish a reserve of undifferentiated stem cells. Adapted from Sassone-Corsi 1997.

2.3.4 Genetic Causes for Primary Testicular Failure and Male Infertility

2.3.4.1 Syndromic Primary Testicular Failure

There are relatively few established genetic causes of male infertility. The rate of chromosomal anomaly among infertile men, however, is high: an 8-to 10-fold higher prevalence compared to fertile men (Chandley 1998). Chromosomal abnormalities associated with male infertility can be classified as numerical, structural, or those affecting the Y chromosome. Klinefelter syndrome (KS), 47,XXY, is the most prevalent chromosomal defect, accounting for approximately 11% of azoospermic cases (Van Assche et al. 1996). The classic phenotype of KS includes tall stature, gynecomastia, small testes and hypergonadotropic hypogonadism (Klinefelter et al. 1942, Wikström and Dunkel 2011). The phenotype is highly variable, and thus it is estimated that most KS men will never receive diagnosis (Bojesen et al. 2003). The only constant clinical finding in KS is small testes (2-4 ml) (McLachlan and O'Bryan 2010). Histologically, the testes of KS men are characterized by Sertoli-cell-only syndrome and hyalinized tubules (McLachlan et al. 2007). However, many patients have small foci of spermatogenesis that allows the isolation of testicular sperm in 40-69% of nonmosaic KS patients (Friedler et al. 2001, Levron et al. 2000, Schiff et al. 2005, Tournaye et al. 1996). This permits the use of intracytoplasmic sperm injection (ICSI) and enables the possibility of biological offspring for these patients (Palermo et al. 1998). Interestingly, sperm from most KS men have a normal 23X or Y complement (McLachlan and O'Bryan 2010).

Bloom syndrome (OMIM 210900) is an autosomal recessive disorder caused by mutations in the *BLM* (Bloom syndrome, RecQ helicase-like) gene on chromo-

some 15q26.1 (Ellis et al. 1995). The corresponding protein is a nuclear cell cycle regulator, deficiency of which leads to hypermutability (Ellis and German 1996, Auerbach and Verlander 1997). Bloom syndrome is characterized by intrauterine growth restriction (IUGR), sunlight sensitivity leading to telangiectasia erythema, immunologic deficiency, and an increased risk for neoplasia. Males are infertile and usually present with azoospermia and small testes (Bloom 1954, 1966). About 150 patients are known to exist, and the carrier rate is approximately 1% among Ashkenazi Jews (Passarge 1991, Roa et al. 1999). In contrast, female patients with Bloom syndrome report variable hypogonadism but are fertile (Hall 2010).

Noonan syndrome (NS; OMIM 163950) is a quite common multiple malformation syndrome with an estimated incidence of 1 in 500 (Noonan 1994). About 50% of patients have mutations in the *PTPN11* (protein tyrosine phosphatase, non-receptor type 11) gene (Tartaglia et al. 2001). NS has a wide phenotypic variation but typical features include characteristic facies, short stature and congenital heart disease, commonly pulmonary stenosis (Noonan 1994). Male patients typically have delayed puberty (Noonan 2006) and undescended testes (Sharland et al. 1992). Although male transmission of NS is seen, fertility is impaired (Allanson 2007). This was previously largely attributed to cryptorchidism, well known to be associated with compromised fertility (Werder et al. 1976, Cortes et al. 2003). However, recent studies indicate that primary testicular failure, with both Sertoli and Leydig cell dysfunction, may underlie the impaired fertility seen in NS males (Marcus et al. 2008, Ankarberg-Lindgren et al. 2011). Female patients with NS typically have delayed puberty but fertility does not seem to be impaired (Allanson 2007).

Prader-Willi syndrome (PWS; OMIM 176270) is a complex multisystem disorder characterized by short stature, hyperphagia leading to morbid obesity, muscular hypotonia, developmental delay, and hypogonadism (Gunay-Aygun et al. 2001, Cassidy and Driscoll 2009). The hypogonadism has been traditionally regarded as hypothalamic, but recent evidence suggests that the hypogonadism in fact may be central, peripheral, or combined (Eiholzer et al. 2006, Radicioni et al. 2012). PWS results from lack of expression of the paternally inherited genes in the PWS critical region on chromosome 15 (Buiting 2010). Genital hypoplasia, cryptorchidism, and incomplete pubertal development are common among male patients (Crinò et al. 2003). Fertility has never been reported in PWS males, whereas females with PWS demonstrate variable hypogonadism and pregnancies have been reported (Eldar-Geva et al. 2010).

2.3.4.2 Non-Syndromic Testicular Failure

Chromosome Yq microdeletions are the most prevalent identifiable genetic cause of spermatogenic failure, accounting for about 10% in non-obstructive azoospermic men and 3-5% in idiopathic severe oligozoospermic men (Krausz

and Degl'Innocenti 2006). These deletions yield partial or complete removal of one or more of the three AZF (azoospermia factor) regions; AZFa, AZFb and AZFc, which encode multiple genes involved in spermatogenesis (Vogt et al. 1996). In each AZF region, candidate genes have been identified, but their role in spermatogenesis remains largely unknown (Skaletsky et al. 2003). The vast majority of these microdeletions arise *de novo*, denoting that this area is especially unstable.

Androgen receptor (AR) gene mutations give rise to androgen insensitivity syndrome (AIS; OMIM 300068), an X-linked disorder leading to end-organ androgen resistance. More than 800 different mutations in the *AR* gene have been reported. AIS affects sexual differentiation with varying severity, depending on the type and localization of the mutations, ranging from a female phenotype (complete AIS; CAIS) to simple defective spermatogenesis in otherwise normal males (partial AIS; PAIS) (Hiort et al. 1996, 2000). In unselected infertile men, the prevalence of mutations in the *AR* gene varies between 0-1.7% (Ferlin et al. 2006, Rajender et al. 2007).

During the last few years, new gene mutations have been correlated with specific morphological defects of the spermatozoa. For example, mutations in the aurora kinase C gene (*AURKC*) cause meiotic arrest and lead to the production of large-headed polyploid spermatozoa with multiple flagella (Dieterich et al. 2007, 2009). In addition, a complete deletion of the *DPY19L2* (dpy-19-like 2) gene is responsible for most cases of globozoospermia, characterized by the production of round spermatozoa without an acrosome (Harbuz et al. 2011). Mutations in *CATSPER1* (cation channel, sperm associated 1) gene are associated with recessive male infertility (Avenarius et al. 2009), and mutations in the dynein genes that encode proteins of the axonemal dynein cluster are associated with asthenozoospermia. The collective frequency of these mutations in the general population is extremely low, however.

Recently, Bashamboo et al. (2010) showed that mutations in *NR5A1*, a key factor in gonadal and adrenal development, underlie spermatogenic failure in about 4% of otherwise healthy men. *NR5A1* is an important transcriptional regulator of genes involved in the hypothalamic-pituitary-steroidogenic axis (Morohashi et al. 1992, Luo et al. 1994). It is expressed in both Sertoli and Leydig cells during the development of the testis and in Sertoli cells during prepuberty and adulthood (Ikeda et al. 1994, Hanley et al. 1999, Morohashi et al. 1994).

NR5A1 mutations are related to many distinct phenotypes, such as 46,XY partial and complete gonadal dysgenesis, penoscrotal hypospadias, micropenis, and interestingly, 46,XX primary ovarian insufficiency (Lin and Achermann 2008, Lourenço et al. 2009). In Leydig cell-specific *Nr5a1* knockout mice, the testes are hypoplastic and spermatogonia fail to develop into mature sperm

(Jeyasuria et al. 2004). In addition, the mice show diminished expression of two genes essential for testosterone biosynthesis, *Cyp11a* and *Star*. Accordingly, in a study of azoospermic men, expression levels of NR5A1 in testes correlated positively with serum testosterone concentrations, indicating a direct connection between these two factors (Kojima et al. 2006).

2.3.5 Symptoms and Diagnosis of Testicular Failure

Symptoms of testicular failure are determined by the age of onset and genetic factors. In adult males, primary testicular failure is associated with impaired spermatogenesis, low testosterone levels and elevated FSH and LH levels. Consequently, symptoms and signs are due to infertility or androgen deficiency. If the androgen deficiency manifests before the completion of puberty, incomplete sexual development, eunuchoidal body proportions, and retention of a high-pitched voice will follow. Androgen deficiency developing after the completion of puberty is associated with infertility, reduced sexual desire and function, small or shrinking testes, and reduced muscle mass. In addition, non-specific symptoms such as decreased energy and depressed mood can occur (Bhasin and Basaria 2011). In isolated spermatogenic failure, LH and testosterone levels are normal, thus the patient suffers no symptoms of androgen deficiency (Bhasin 2007).

2.3.6 Prognosis of Testicular Failure

In contrast to secondary hypogonadism, where fertility often can be restored by hormonal therapy, in primary hypogonadism, treatment of infertility may be restricted to assisted reproductive techniques, such as intracytoplasmic sperm injection (ICSI), the use of donor sperm, or adoption (Bhasin and Basaria 2011). In young men with androgen deficiency, testosterone treatment is generally favorable with regard to sexual function, energy, body composition, and bone density (Cunningham and Toma 2011).

2.4 Mulibrey Nanism

2.4.1 Clinical Features

Mulibrey nanism (MUL; OMIM 253250) is an autosomal recessive disorder belonging to the Finnish disease heritage and is caused by mutations in the *TRIM37* gene. Perheentupa and colleagues first described the disorder in the early 1970s (Perheentupa et al. 1970, 1973). The name is an acronym from MUscle, LIver, BRein and EYe, indicating distinct findings in these organs, and from nanism, which stands for short stature. Today, 100 patients from Finland and about 40 sporadic cases from the rest of the world are known (Thorén 1973, Cumming et al. 1976, Voorhess et al. 1976, Similä et al. 1980, Finni and Herva 1981, Sánchez-Corona et al. 1983, Cotton et al. 1988, Haraldsson et al. 1993, Lapunzina et al. 1995, Seemanová and Bartsch 1999, Avela et al. 2000, Jagiello et al. 2003, Hämäläinen et al. 2004, 2006, Doğanc et al. 2007). Each year, about two new MUL patients are born in Finland, with an incidence of approximately 1/40 000 (Lipsanen-Nyman 1986). The majority of the Finnish patients come from clustered regions in Savo and North Carelia, in line with the other diseases of the Finnish disease heritage. Genetically, the Finnish patients form a very homogenous group, whereas all patients carry the same founder mutation, named Fin-major, the vast majority in a homozygous form.

The first described MUL patients were characterized by hypoplastic facial features, a prominent forehead, and a J-shaped sella turcica. In addition, they displayed liver enlargement, fibrous dysplasia of long bones, and cutaneous naevi flammei. Nearly all patients carried yellowish dots in the ocular fundi (Raitta and Perheentupa 1974). Constrictive pericarditis and congestive heart failure were characteristic signs already in the first recognized MUL patients (Tuuteri et al. 1974). In addition, a high-pitched voice and a small tongue were apparent, and dental crowding was commonly seen (Myllärniemi et al. 1978). In 1986, a Finnish monography (Lipsanen-Nyman 1986) systematically evaluated the patients known to that date.

Lipsanen-Nyman et al. characterized the Mulibrey heart disease in 2003. They concluded that constrictive pericarditis, myocardial hypertrophy and fibrosis constitute the main factors of the heart disease and that over half of the MUL patients will ultimately develop congestive heart failure (CHF). The heart disease is the main prognostic factor of the patients (Lipsanen-Nyman et al. 2003).



Figure 9. Characteristic facial features in a 2-year-old girl with MUL. Note the frontal bossing, high hairline, low nasal bridge and low-set ears. The pictures are published with permission from the patient and her parents.

In 2004, the clinical characteristics at time of diagnosis were analyzed in the national cohort of 85 Finnish MUL patients (Karlberg et al. 2004). The authors concluded that the clinical features of the Finnish MUL patients form a distinct entity, although organ manifestations significantly differ from childhood to adulthood and between patients. The most consistent attributes were growth failure with characteristic craniofacial features (Figure 9). In infancy, the most frequent problems were failure to thrive with feeding difficulties and respiratory tract infections, including pneumonias. At the time of diagnosis (median age 2.1 years), more than 90% of the patients presented with characteristic facial features (scaphocephaly, facial triangularity, high and broad forehead, and low nasal bridge). Moreover, nearly all patients were gracile and had thin extremities (Figure 10). Other common findings included high-pitched voice (96%), yellowish dots in the ocular fundi (79%), mild muscular hypotonicity (68%), cutaneous naevi flammei (65%), hepatomegaly (45%), and fibrous dysplasia of long bones (25%). Psychomotor development was mostly normal or slightly delayed, but approximately half of the patients showed mild hypotonicity in infancy (Karlberg et al. 2004).

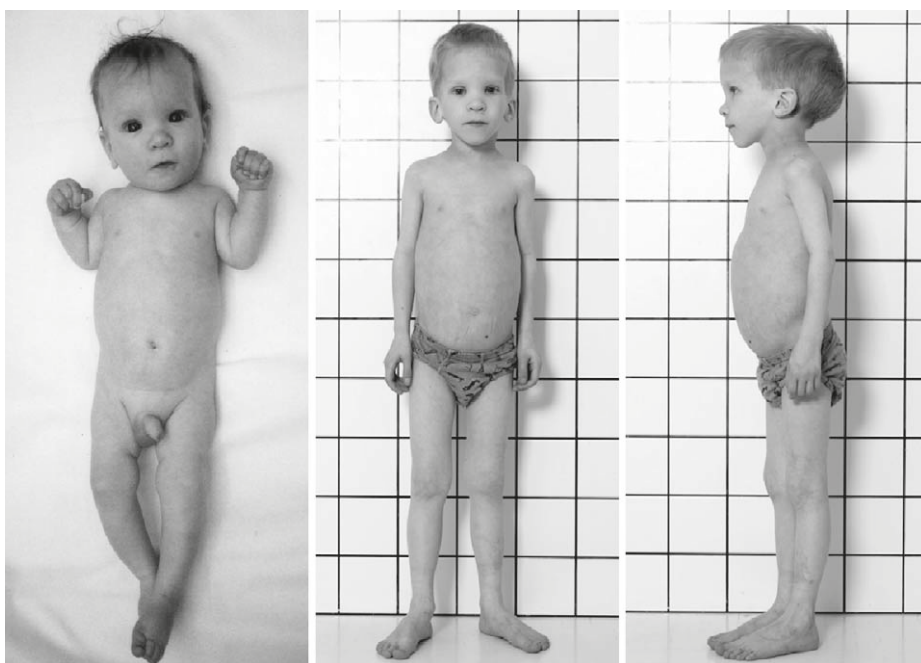


Figure 10. A boy with MUL at age 4 months and 7.5 years. Note the gracile body with thin extremities. The pictures are published with permission from the patient and his parents.

2.4.2 Growth in MUL

MUL children are born small (short and light) for gestational age (SGA) after a median gestation length of 39 weeks (32-42 weeks). Most of the pregnancies proceed without substantial complications, although poor fetal growth is often observed. Adjusted to 40 weeks of gestation, the median birth length for girls is 44.8 cm (median hSDS -3.0) and birth weight is 2300g (median SDS -3.0). For boys, the median birth length is 45.0 cm (median hSDS -2.8) and weight 2350g (median SDS -2.9) (Karlberg et al. 2004). In contrast to SGA due to other reasons (Gibson et al. 2003), MUL children fail to catch up in growth within 48 months after birth. Instead, they show a continuous deceleration in both relative height and weight during infancy and early childhood, followed by a spontaneous, but incomplete catch-up growth up to school age. The pubertal growth spurt is typically weak or nonexistent. GH therapy improves prepubertal growth, but has only modest impact on adult height (average +5cm). The average final adult height is 136 cm (median hSDS -5.1) in females and 150 cm (median hSDS -4.1) in males (Karlberg et al. 2007).

2.4.3 Metabolic Features of MUL

With increasing age, MUL patients experience a pronounced change in glucose metabolism. While children less than 10 years have low fasting glucose and insulin levels, over 90% of the adults display insulin resistance (IR). Of these, 42% have impaired glucose tolerance (IGT) and 50% have type 2 diabetes, developing at a median age of 18.3 years and 26.0 years, respectively. The development of IR can also be observed in the patients' body composition; while the children are slim, abdominal obesity begins to develop after puberty and 42% of the adults are overweight (weight for height >20%). In addition, the development of IR is accompanied by changes in lipid metabolism, manifesting as increasing levels of total cholesterol, triglycerides, serum leptin, and uric acid with age (Karlberg et al. 2005). GH is an insulin antagonist and may induce hyperinsulinemia, but intriguingly, patients treated with GH are slimmer and have less metabolic problems as young adults (Karlberg et al. 2007).

Fatty liver is evident in the majority of adults and adolescents, in addition to almost half of the reported prepubertal MUL children. Of all MUL adults, more than 80% present with hypertension (>95th percentile for age and sex). Accordingly, the majority of adults (70%) fulfil the National Cholesterol Education Program (NCEP) criteria for metabolic syndrome (Karlberg et al. 2005). The pathophysiology behind the development of IR in MUL is unclear. IUGR itself is associated with an increased risk of IR explained by an unfavorable mother-child environment followed by a rapid early postnatal weight gain (Veening et al. 2003, Eriksson et al. 2003). In MUL, however, the postnatal weight gain is very modest and the underlying cause of the growth defect is genetic.

2.4.4 Diagnosis of MUL

The diagnosis of MUL remains challenging. There is an abundance of conditions leading to intrauterine growth restriction (IUGR), including underlying genetic disease of the fetus, placental insufficiency or infections. The combination of prenatal onset growth failure, dysmorphic facial features, poor weight gain, and hepatomegaly should evoke suspicion of MUL in early infancy, however (Karlberg et al. 2004). The median age at diagnosis of the Finnish MUL patients is 2.1 years. In 2004, revised diagnostic criteria were proposed for MUL (Table 1). Although none of the features are constant findings in the Finnish MUL patients, 99% of the patients presented with at least three of the major signs, and two of the minor signs (Karlberg et al. 2004). Three unusually mild cases of MUL have been diagnosed among the Finnish patients. All three are homozygous for the Fin-major mutation, indicating that the phenotype in MUL is variable. Since the identification of the causative *TRIM37* gene in the year 2000, genetic confirmation of the diagnosis has been possible. For Finnish families, screening for the Fin-major mutation is often sufficient. For non-Finn-

ish patients, however, sequencing of the whole *TRIM37* gene is necessary. In affected families, prenatal genetic counselling is plausible.

Table 1. Diagnostic criteria and their prevalence in MUL

Signs	Frequency (%)
Major signs	
1. Growth failure (A or B or C)	
A) SGA without catch-up growth	95
B) height in children 2.5 SD below population mean for age	94
C) height in adults 3.0 SD below population mean	90
2. Characteristic radiological findings (A or B)	
A) slender long bones with thick cortex and narrow medullar channels	93
B) J-shaped (low and shallow) sella turcica	89
3. Characteristic craniofacial features	90
Scaphocephaly, triangular face, high and broad forehead, low nasal bridge and telecanthus	
4. Characteristic ocular findings	79
Yellowish dots in retina	
5. Mulibrey nanism in a sibling	17
Minor signs	
1. High pitched voice	96
2. Hepatomegaly	70
3. Cutaneous naevi flammei	65
4. Fibrous dysplasia of long bone	25

Three major signs + 1 minor sign or 2 major signs + 3 minor signs are required for diagnosis. Adapted from Karlberg et al 2004

2.4.5 Differential Diagnosis

Disorders with prenatal onset growth failure and dysmorphic craniofacial features constitute the main components in the clinical differential diagnosis of MUL, Silver-Russell syndrome (SRS) representing the most important of them. SRS and MUL patients have a very similar growth pattern, with prenatal onset growth restriction and lack of postnatal catch-up growth. In addition, SRS patients also present with facial dysmorphism, constitutional gracility, and feed-

ing difficulties in infancy. These two conditions have several distinctive features, however. SRS is characterized by clinodactyly (curving of the fifth digit), small face with prominent triangularity, micrognathia (small jaws) with downturned mouth corners, and skeletal asymmetry (Price et al. 1999, Hannula et al. 2001, Hitchins et al. 2001), features uncommon in MUL. On the other hand, hepatomegaly, fibrous dysplasia, heart involvement and yellow dots in the ocular fundi, which are typical in MUL, are not seen in SRS. However, SRS exhibits marked genetic and clinical diversity (Hannula et al. 2001, Hitchins et al. 2001, Abu-Amro et al. 2008, Bruce et al. 2008). The classical features of SRS that clinically overlap with MUL are associated with hypomethylation of the H19 imprinting control region (ICR) on chromosome 11p15.5 (Bruce et al. 2009). Both MUL and SRS should be kept in mind when evaluating an infant with growth restriction and facial dysmorphism.

2.5 *TRIM37* Gene

The gene underlying MUL, *TRIM37*, was identified in the year 2000 by positional cloning. The project had been initiated already in the early 1990s (Avela et al. 1997, 2000). In MUL patients, this strategy identified four different mutations in a cDNA sequence (KIAA0898; 4111bp) encoding for a previously unknown member of the RING-B-Box-Coiled-Coil (RBCC) protein family (Avela et al. 2000). The KIAA0898 gene was located on chromosome 17q22-23 and was named *MUL*, referring to Mulibrey nanism. The gene was subsequently renamed *TRIM37*, however, while the corresponding protein, TRIM37, was included in the newly characterized TRIM protein family (Reymond et al. 2001).

To date, 19 different disease-associated mutations have been identified in the *TRIM37* gene (Avela et al. 2000, Jagiello et al. 2003, Hämäläinen et al. 2004, 2006, Kallijärvi et al. 2005, Doğanc et al. 2007, and five unpublished mutations). Of the Finnish MUL patients, 95% are homozygous for the Fin-major mutation (c.493-2A>G). This mutation results in aberrant splicing resulting in a deletion of five nucleotides from the final mRNA transcript. The Fin-minor mutation (c.2212delG) has thus far been detected in only three Finnish patients, who are compound heterozygotes for the Fin-major and Fin-minor mutations. Two additional mutations have been identified in three Finnish patients (c.227T>C in one patient and c.1166A>G in two siblings), and these mutations were observed together with the Fin-major mutation in compound heterozygote forms (Kallijärvi et al. 2005, Hämäläinen et al. unpublished data). So far, all identified *TRIM37* mutations seem to produce loss-of-function alleles and a non-functioning protein. Thus, there is no evidence of a genotype-phenotype correlation in MUL.

2.6 TRIM37 Protein

TRIM37 is a typical member of the TRIM protein family, with the N-terminal TRIM structure comprising the RING domain, a single B-box domain, and a coiled-coil region (Figure 11). An internal TRAF (tumor necrosis factor (TNF) receptor associated factor) domain follows the TRIM domain (Zapata et al. 2001). The C-terminal part of TRIM37 has no recognized functional domains and is also the least conserved part of the protein (Avela et al 2000). TRAF family proteins are commonly involved in TNF receptor signaling and function as adaptor proteins for a diversity of cell surface receptors, involved in regulating apoptosis, cell survival, and cellular stress responses (Bradley et al. 2001).

Northern blot analysis proves that *TRIM37* mRNA is widely expressed in adult human tissues, with the highest levels in testis and brain (Avela et al. 2000). *TRIM37* mRNA expression is also broadly detected in many tissues during human and mouse embryogenesis. Particularly high expression is seen in dorsal root and trigeminal ganglia, liver, and epithelia of several organs.

Like several other RING proteins (Borden 2000), TRIM37 also possesses ubiquitin E3-ligase activity, although the precise biological function of the protein is still unknown (Kallijärvi et al. 2005).

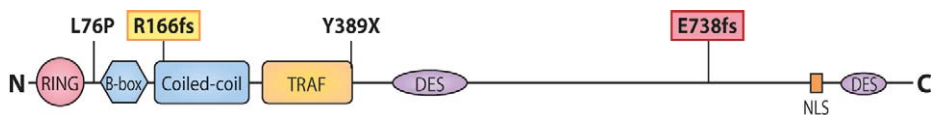


Figure 11. A schematic structure of the TRIM37 protein. The four Finnish mutations are depicted; the Fin-major mutation in the yellow box and the Fin-minor in the pink box.

2.6.1 TRIM37 and Peroxisomes

MUL is currently classified as a peroxisomal disorder, due to the fact that TRIM37 localizes to peroxisomes in immunofluorescence studies (Kallijärvi et al. 2002). No known peroxisomal targeting signals have been identified in TRIM37, however, which means that the localization may be contingent on interaction partners. The coiled coil region of TRIM proteins is sufficient and crucial for their correct localization to subcellular compartments (Reymond et al. 2001). Accordingly, the Fin-major mutation disrupts the coiled coil region and subsequently, mutant TRIM37 loses its peroxisomal localization. On the other hand, the coiled coil region remains intact as a result of the Fin-minor mutation and the mutant protein targets correctly to the peroxisomes (Kallijärvi et al. 2002).

Peroxisomes are cellular organelles with an imperative role in the metabolism of lipids and other essential biomolecules (Wanders and Waterham 2006). Until recently, peroxisomes were regarded as autonomous organelles, but emerging evidence show that peroxisomes have functional and physical interactions with other cellular organelles, for example with mitochondria. Peroxisomes and mitochondria collaborate closely in several metabolic pathways, including β -oxidation of fatty acids and the metabolism of reactive oxygen species (ROS) (Schrader and Yoon 2007, Thoms et al. 2009).

Peroxisomal disorders can be divided into two groups, peroxisomal biogenesis disorders (PBDs) and single peroxisomal protein defects (Weller et al. 2003). PBDs are severe, autosomal recessive disorders caused by mutations in the *PEX* genes. These mutations result in failure to assemble the organelle, and lead to defects of multiple peroxisome functions (Thoms et al. 2009). PBD patients have a progressive metabolic disease and developmental disorder. The clinical picture includes facial dysmorphism, skeletal, liver, and ocular abnormalities, and neurological symptoms including muscular hypotonia, failure to thrive, seizures, and slow or absent psychomotor development (Weller et al. 2003). One of the most severe PBDs is Zellweger syndrome (ZW, OMIM 214100), in which patients suffer from a severe neurodevelopmental disorder and, in the classical form, seldom survive the first year of life (Steinberg et al. 2006).

Single peroxisomal protein defects lead to deficiency of a specific peroxisomal function and are either autosomal recessive or X-chromosomal recessive. X-linked adrenoleukodystrophy (X-ALD, OMIM 300100), resulting from alterations in the *ABCD1* gene, is the most prevalent single protein defect. X-ALD results in defective peroxisomal β -oxidation and subsequent accumulation of very long chain fatty acids (VLCFAs) in all tissues, primarily affecting the adrenal cortex and the nervous system. The phenotype is highly variable and ranges from asymptomatic to severe neurological deterioration in childhood, with varying degrees of adrenal insufficiency (Gärtner et al. 1998, Moser et al. 2007, Thoms et al. 2009).

MUL is not a typical member of the peroxisomal disease spectrum. Although some features are shared, especially with the PBDs, other characteristic findings of peroxisomal disorders, such as neurological abnormalities including mental retardation, are rarely seen in MUL. Facial dysmorphism, growth restriction, retinal changes, muscular hypotonia and hepatomegaly are seen in both conditions, however. In fact, peroxisomal function has previously been evaluated in two MUL patients, revealing no biochemical evidence of major peroxisomal dysfunction (Schutgens et al. 1994). Nevertheless, MUL is currently regarded as a peroxisomal disorder.

2.7 TRIM Protein Family

Members of the family of tripartite motif (TRIM)-containing proteins are characterized by a RING finger domain, one or two zinc-binding motifs (named B-boxes) and an associated coiled coil region (Reymond et al. 2001). The TRIM structure is highly conserved in order and spacing, suggesting a common general function to the tripartite motif proteins. The RING domain is consistently found in the N-terminus of these proteins, whereas the C-terminus contains variable domains. Today, more than 70 TRIM proteins have been identified in humans and mice, encoded by approximately 71 genes in man (Hatakeyama 2011). Many of these genes are clustered together, for example in addition to *TRIM37*, the long arm of chromosome 17 also carries *TRIM25*, *TRIM47*, and *TRIM65*. TRIM proteins can further be divided into subfamilies, I to XI, on the basis of differences in their domain structure (Short and Cox 2006); *TRIM37* is the sole member of subfamily VIII. TRIM proteins may form homodimers, heterodimers, or even trimers, suggesting that TRIM proteins can vary their substrate specificity by changing their binding partner (Reymond et al. 2001, Herquel et al. 2011).

TRIM family proteins are involved in an extensive range of biological processes, including transcriptional regulation, development, cell growth, apoptosis, and tumorigenesis. Accordingly, alterations in TRIM proteins are associated with diverse pathological conditions, such as developmental disorders, viral infections, and cancer (Ozato et al. 2008, Mc Nab et al. 2011, Meroni and Diez-Roux 2005).

2.7.1. TRIM Proteins in Cancer

Several TRIM family proteins are involved in carcinogenesis and cancer progression (Table 2). For example *TRIM25*, the corresponding gene (*TRIM25*) of which is located in the same cluster as *TRIM37* on chromosome 17, has been implicated in ovarian, breast, and endometrial cancer. The expression of *TRIM25* is induced by estrogen, and *TRIM25* protein is abundantly expressed in the female reproductive organs, as well as in breast and ovarian cancer. *TRIM25* mediates the ubiquitination and degradation of 14-3-3 sigma, which is a negative cell cycle regulator that causes cell cycle arrest (Urano et al. 2002). Loss of *Trim25* in mouse embryonic fibroblasts causes an accumulation of 14-3-3 sigma, leading to reduced cell proliferation. *Trim25*^{-/-} mice are viable and fertile, but the uteri of *Trim25*^{-/-} female mice are markedly underdeveloped, suggesting that *TRIM25* is imperative for estrogen-mediated cell proliferation in the uterus (Orimo et al. 1999, Hatakeyama 2011).

The RING domain is strongly associated with ubiquitination (Joazeiro and Weissman 2000), and today it is recognized, that the majority of TRIM proteins function as E3 ubiquitin ligases (Borden 2000, Hatakeyama 2011). For example,

the tumor suppressor gene *BRCA1* encodes for a RING finger E3 ligase BRCA1. Germline mutations in *BRCA1* predispose women to early onset breast and ovarian cancer (Starita and Parvin 2003, Mani and Gelmann 2005).

Table 2. TRIM proteins implicated in cancer

Protein	Cancer type
TRIM8	Glioblastoma
	Laryngeal cancer
TRIM13	B cell chronic lymphocytic leukaemia
	Chronic lymphocytic leukaemia
TRIM19	Acute promyleocytic leukaemia
TRIM24	Papillary thyroid cancer
	Myeloproliferative syndrome
	Liver cancer
	Myelodysplastic syndrome-related acute myeloid leukaemia
	Breast cancer
TRIM25	Ovarian cancer
	Breast cancer
	Endometrial cancer
TRIM27	Lymphoma
	Breast cancer
TRIM28	Gastric cancer
TRIM29	Lung cancer
	Bladder cancer
	Colon cancer
	Ovarian cancer
	Endometrial cancer
	Multiple myeloma
	Gastric cancer
TRIM31	Gastric cancer
TRIM32	Head and neck cancer
TRIM33	Chronic myelomonocytic cancer
TRIM40	Colon cancer
TRIM68	Prostate cancer

Adapted from Hatakeyama 2011

2.8 Ubiquitin-Proteasome System

Ubiquitination is a post-translational modification of proteins in the eukaryotic cell essential for maintaining protein homeostasis (Hershko and Ciechanover 1982, 1992, 1998). In this highly conserved process, a single molecule or multiple molecules of the 76-amino acid ubiquitin are covalently conjugated to target proteins, thus marking them for degradation. This proteolytic pathway is pivotal in the elimination of short-lived regulatory proteins, including those that participate in cell cycle regulation, cellular signaling, DNA repair, morpho-

genesis, protein quality control and transcriptional regulation (Hatakeyama 2011). This pathway also eliminates inappropriately folded, unassembled, oxidized, or otherwise damaged proteins that possibly could form toxic aggregates in the cell.

The process of ubiquitination involves three steps, each catalyzed by specific enzymes, namely ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) (Pickart 2001b) (Figure 12). Usually, the ubiquitin process is repeated, resulting in a polyubiquitin chain, linked by different lysine residues of ubiquitin (Pickart 2001b). Substrates marked with a Lys48-linked polyubiquitin chain are usually subsequently targeted to the 26S proteasome for destruction, whereas monoubiquitinated or Lys63-linked polyubiquitinated substrates mainly regulate a diversity of cellular functions, including endocytosis, transcription, and DNA repair (Weissman 2001, Hicke and Dunn 2003, Mukhopadhyay and Riezman 2007).

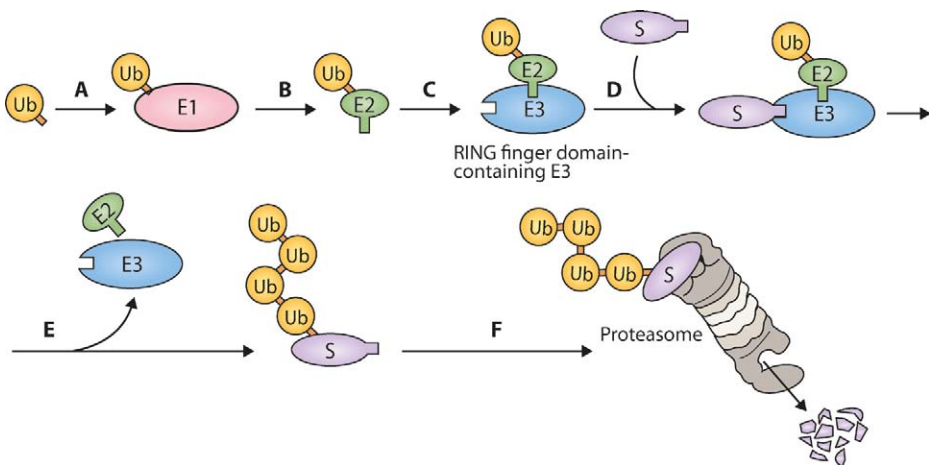


Figure 12. The ubiquitin-proteasome system. A) Ubiquitin (Ub) is activated by the ubiquitin-activating enzyme (E1) using ATP. **B)** The activated ubiquitin is transferred and bound to the ubiquitin-conjugating enzyme (E2). **C)** The ubiquitin ligase (E3) recognizes the specific E2. **D)** E3 tags the specific target substrate (S) with ubiquitin. **E)** The ubiquitin process is usually repeated generating a polyubiquitin chain. **F)** Finally the polyubiquitinated substrate is targeted to the 26S proteasome for degradation. Modified from Weissman et al. 2011.

The E3-ligase is thought to be the component of the ubiquitin conjugation pathway that is most directly responsible for substrate recognition, whereas the E3 proteins function as scaffold proteins mediating the interaction between E2 and the substrate (Hershko and Ciechanover 1998, Scheffner et al. 1995). The E3 ligases can thus be named the “quality controllers” of the ubiquitin-proteasome system (UPS). Each E3 ligase recognizes a unique set of substrates, together with a single or few E2 ligases (Borden 2000, Pickart 2001a,b). Some substrates can also be targeted by more than one E3 ligase (Mani and Gelmann 2005). It is estimated that the human genome contains approximately 1000 E3 ligases, but only a few have been characterized and their substrates recognized (Schwartz and Ciechanover 2009). There are two major families of E3 ligases: the E3s containing a HECT (homologous to E6-AP C terminus domain), and E3s containing a RING-finger domain (Weissman 2001).

2.8.1 Ubiquitin-Proteasome System in Disease

By degrading mutant and structurally abnormal proteins, the UPS plays a crucial role in maintaining homeostasis and normal function in eukaryotic cells (Paul 2008). Abnormalities in the UPS have been implicated in the pathogenesis of several inherited and acquired disorders. The pathologies may result from gain or loss of function. In gain of function, a substrate meant for degradation is stabilized due to inactivation of an enzyme in the UPS pathway, or from a mutation in a targeting motif in the substrate. In loss of function, the substrate is removed in an uncontrolled and accelerated way (destabilized). Subsequently, impaired function of the UPS has been linked to the pathogenesis of several genetic, autoimmune, and neurodegenerative disorders (Paul 2008). Furthermore, there is increasing evidence that the UPS may play a central role in the pathogenesis of many malignancies.

Dysfunction of proteosomal degradation can either enhance the effect of oncoproteins (by stabilization) or reduce the quantity of tumor suppressor proteins (by destabilization) (Mani and Gelmann 2005, Paul 2008). Some of the substrates normally degraded by the UPS, like c-Myc, c-Fos and c-Jun, are oncoproteins, which may induce malignant transformation if not appropriately removed from the cell. Mutation or translocation of c-Myc is associated with B-cell lymphomas (Leder et al. 1983). c-Fos and c-Jun have both been implicated in colorectal cancer (Milde-Langosch 2005).

Destabilization of tumor suppressor proteins, such as p53, by an over-activated UPS, has also been implicated in tumorigenesis (Paul 2008). The p53 gene, located on chromosome 17p13.1, is the most prevalent target in human cancers (Hainaut and Hollstein 2000, Hollstein et al. 1991). The p53 protein is a transcription factor, which responds to DNA damage and stress by activating cell cycle arrest and apoptosis to avoid accumulation of damaged cells (Levine 1997). p53 is regulated by the E3 ubiquitin ligases MDM2 (RING-type E3 ligase) and

E6-AP (HECT-type E3 ligase) (Burger and Seth 2004). Normally, MDM2 regulates the basal levels of p53 by targeting it for proteosomal degradation (Honda et al 1997, Haupt et al 1997). Amplification of the *MDM2* gene, found for example in sarcomas, lipomas and liposarcomas, results in overexpression of MDM2 and causes downregulation and subsequent inactivation of p53 (Momand et al 1998).

In addition, TRIM proteins, including TRIM13, TRIM19, and TRIM24, are involved in p53-regulated pathways, such as apoptosis and response to DNA damage. For example, TRIM24 regulates the ubiquitination of p53 and negatively controls p53 levels. The *TRIM24* gene is overexpressed in breast cancer, and deletion of *TRIM24* causes p53-dependent apoptosis, indicating that TRIM24 could be a potential therapeutic agent in breast cancers that express wild-type p53 (Tsai et al. 2010, Chambon et al. 2011, Hatakeyama 2011).

Indeed, the hierarchical nature of the UPS provides many targets for specific intervention and has accordingly arisen as a promising approach to new anti-cancer therapies (Burger and Seth 2004). Bortezomib is a proteasome inhibitory agent that is already used for the treatment of multiple myeloma (Hideshima et al. 2001). Moreover, drugs targeting individual E3 ligases are even more promising, since they should provide higher levels of sensitivity, as they would be more likely to prevent binding to specific substrates. Drugs that inhibit oncogenic TRIM proteins could also prove useful in the future (Hatakeyama 2011).

The von Hippel-Lindau protein (pVHL) functions as a ubiquitin E3 ligase, and is part of the VHL ubiquitin ligase complex that regulates the proteasomal degradation of hypoxia-inducible factor-1 α (HIF-1 α) under normal oxygen conditions. HIF-1 α normally mediates physiologic responses to hypoxia, by activating the transcription of genes that promote angiogenesis, like the vascular endothelial growth factor (VEGF). The von Hippel-Lindau tumor suppressor gene (*VHL*) on chromosome 3p25 is mutated in the autosomal dominant cancer syndrome von Hippel-Lindau (VHL; OMIM 193300) (Latif et al. 1993). In this syndrome, patients develop multiple tumors including renal cell carcinoma, hemangioblastomas, and pheochromocytomas, in addition to cystic changes in multiple organs (Kaelin 2002, 2007). Loss of pVHL function leads to a constitutive activation of HIF-1 α , resulting in an increased expression of VEGF, for example, promoting tumor growth and angiogenesis, and thus predisposing patients to hypervascular lesions in different organs (Clifford and Maher 2001, Jung et al. 2006). Although it is quite a restricted set of tissues that develop tumors in VHL disease, the *VHL* gene is ubiquitously expressed in most tissues (Los et al. 1996, Kaelin 2007). *VHL* mutations are relatively common in sporadic renal cell carcinoma and sporadic hemangioblastomas (Kim and Kaelin 2004). Apart from renal carcinomas, somatic *VHL* mutations are rarely seen in other epithelial cancers, however, occasionally, somatic *VHL* mutations have been described in colorectal cancer (Giles et al. 2006).

3. AIMS OF THE STUDY

Mulibrey nanism (MUL) is a monogenic peroxisomal disorder with diverse organ manifestations caused by mutations in the *TRIM37* gene. The aim of this thesis was to study the sexual maturation and fertility of a well-characterized population of MUL patients, and to characterize the tumors associated with this disorder with a special emphasis on the gynecological tumors. A further aim was to evaluate the possible role of TRIM37 in the development of sporadic gynecological tumors of the thecoma/fibroma type.

More specifically, the aims were as follows:

1. To evaluate the sexual maturation and fertility of MUL women.
2. To study the pubertal development, gonadal function, and fertility of MUL boys and men.
3. To characterize the tumors associated with MUL using histopathology and immunohistochemistry.
4. To study gynecological tumors in MUL and analyze the role of TRIM37 in the development of sporadic fibrothecomas.

4. PATIENTS AND METHODS

The clinical study of MUL patients, on which this study is based, was initiated in year 2000. However, the clinical care of the Finnish MUL patients has primarily been centralized to the Children's Hospital since the 1970s, thus generating a large amount of retrospective data, concomitantly analyzed in this study.

Table 3. Materials and methods used in the study

Material/Method	Publication
Patients	I, II, III, IV
Laboratory examinations	I, II
Semen analysis	II
Radiological studies	I, III
Histology	II, III, IV
Immunohistochemistry	II, III, IV
Mutation analysis	IV
LOH-analysis	IV
Methylation studies	IV

4.1 Patients (I, II, III, IV)

The complete study cohort included 92 Finnish MUL patients (0.7-77 years of age) diagnosed before 2009, 20 of whom were deceased. All patients met the diagnostic criteria for MUL (Karlberg et al. 2004). Of the patients, 86 were homozygous for the Fin-major mutation (c.493-2A>G) of the *TRIM37* gene (Avela et al. 2000) and three patients were compound heterozygotes; two for the Fin-major/Fin-minor (c.2212delG) mutations and one for the c.227T>C/Fin-major mutation (Kallijärvi et al. 2005). The diagnoses had been confirmed by genetic testing in all but three patients. In two of them, DNA was obtained from family members, who were carriers of the Fin-major mutation. The phenotype of the third patient left no doubt about the diagnosis.

In study I, 22 postpubertal women, aged 16-69 years, were studied. Of the women, 15 were included in a clinical study and the data on seven women (five deceased and two with hystero-salpingo-oophorectomy) were studied only retrospectively. Of the patients: 21 were born SGA; 20 had a genetically confirmed diagnosis; 19 were homozygous for the Fin-major mutation, and; 1 was compound heterozygote for the Fin-major and Fin-minor mutations. Of the women in the clinical study, 9/15 had received GH.

The study cohort in study II included 28 male patients aged 8.7 to 50.0 years at the end of the study (9 children and 19 adults, median age 28.8 years), who had regular follow-up at the Children's Hospital from years 2000/2001 to 2010. Of the males: 25 were born SGA and all diagnoses had been confirmed genetically; 26 were homozygous for the Fin-major mutation; 1 was compound heterozygote for the Fin-major and Fin-minor mutations, and; 1 patient had the c.227T>C/Fin-major genotype. One patient died during the follow-up at the age of 14.0 years, 11 males had received GH for a median duration of 9.8 years, and none had received androgen replacement therapy.

Study III included the whole study cohort and study IV included the same patient population as study I.

4.2 Principles of Follow-Up and Clinical Data Collection (I, II, III, IV)

Children were followed up at 6-12 month intervals (II). Visits included physical examination with assessment of height, weight, and pubertal stage according to the criteria of Tanner (Tanner and Whitehouse 1979). The length and width of the testes were measured with a ruler to the nearest mm. Testicular volume was calculated applying the formula $0.52 \times \text{length} \times (\text{width})^2$ (Hansen and With 1952), using the mean value of the two testes for analyses. In study II, reproductive hormone analyses including serum FSH, LH, testosterone, and inhibin B were performed once during childhood (age 2-8 years) and prepuberty (9-11 years), and once a year after the onset of puberty. From the onset of puberty, a GnRH stimulation test was carried out every second year or by clinical indications. Adults were examined at 2-5 year intervals. In study I, serum FSH, LH, inhibin B, estradiol, testosterone, and sex hormone-binding globulin (SHBG) were measured. The blood sample was taken during the early follicular phase from regularly menstruating women, and after a wash-out period of at least six weeks in women receiving hormonal substitution. At each visit, in addition to the physical examination, sexual function, and in women, menstrual history and gynecological symptoms were recorded (I, II).

The hospital records of all patients were retrospectively analyzed and medical history, including laboratory and radiological findings, were recorded (I, II, III, IV).

4.3 Laboratory Examinations (I, II and Unpublished Data)

Serum concentrations of FSH, LH and SHBG were measured by time-resolved immunofluorometric assays (AutoDELFIA™, Wallac, Turku, Finland) (I, II). For FSH, the detection limit was 0.05 IU/liter and the inter-assay coefficient of variation (CV) was $\leq 5\%$ in the concentration range 2-78 IU/liter. Similarly, the detection limit for LH was 0.05 IU/liter and the inter-assay CV was $< 4\%$ in the concentration range 0.3-42 IU/liter. FSH and LH levels measuring less than 0.1

IU/liter were assessed as 0.1 IU/liter. GnRH testing was carried out by giving an intravenous bolus of 100µg GnRH (II). FSH levels were measured at 0, 30, 60, and 90 min, and the LH levels at 0, 20, 30, and 60 min. Estradiol concentrations were assessed by a radioimmunoassay with a sensitivity of 24 pmol/liter (I). Testosterone concentrations were quantified by a radioimmunoassay after separation of the steroid fractions on a Lipidex-5000 microcolumn with a detection limit of 0.1 nmol/liter (I, II). After the year 2005, testosterone concentrations were assayed utilizing a liquid chromatography-tandem mass spectrometric (LC-MS/MS) method (II). The detection limit was 0.15 nmol/liter and the interassay CV was 4.2-7.6%. Serum inhibin B levels were measured by ELISA (Serotec, Oxford, UK), with an assay sensitivity of 15.6 pg/ml, an interassay CV of <15% and an intra-assay CV of <5% (I,II). Normal reference ranges were attained from the Helsinki University Central Hospital Laboratory except for inhibin B in study II, in which reference values were adapted from Andersson et al. 1997.

4.4 Semen Analysis (II)

A semen analysis was offered to all male patients over 16 years of age or turning 16 during follow-up (n=20). Seventeen men provided a semen sample by masturbation after a recommended three days of sexual abstinence and samples were analyzed within 1 h of collection. Basic semen parameters, including semen volume, sperm concentration, sperm motility, and sperm morphology were evaluated according to the 1999 World Health Organization (WHO) guidelines (Table 4). The Sperm Laboratory of Helsinki University Central Hospital performed all semen sample analyses apart from one sample analyzed in the Tampere University Hospital.

Table 4. WHO nomenclature for semen quality

Name	Description
Aspermia	No ejaculate
Azoospermia	Absence of sperm in ejaculate
Oligozoospermia	< 15 x 10 ⁶ sperm/ml of semen
Asthenozoospermia	< 32% progressively motile sperm
Oligoasthenozoospermia	Reduced number and motility of sperm
Teratozoospermia	Increased number of sperm with abnormal morphology

4.5 Radiology (I, III)

Ultrasound (US) of the abdomen was performed regularly as a part of the clinical follow-up of all patients (III). In addition, US of the thyroid gland was carried out in adults (III). If suspicion of a tumor was evoked, a magnetic resonance imaging (MRI) scan was performed (III). Fifteen postpubertal women were subjected to a gynecological US examination which was performed either transvaginally or transrectally utilizing a 5 MHz transducer (Hitachi 520) (I).

4.6 Histology and Immunohistochemistry (II, III, IV)

Tissue specimens were acquired in surgery or from percutaneous core needle biopsies or autopsies (n=17). In study II, testicular specimens from eight patients (aged 2-28) were obtained at orchiectomy (n=2), testicular biopsy (n=1), or autopsy (n=5). Control specimens consisted of four testicular biopsies taken on clinical grounds from 5-30 year old male patients without MUL. Formalin-fixed, paraffin-embedded samples were utilized for the histological and immunohistochemical studies.

The tissue expression of endothelial cell markers CD34 (DakoCytomation, Glostrup, Denmark), CD31 (PECAM-1; Dako), α -smooth muscle actin (α -SMA; Dako), perivascular epithelioid cell (PEC) marker HMB-45 (Dako), cellular proliferation marker MIB-1 (KI67; Dako), and the spermatogonia marker MAGE-A (kind gift from Dr. Giulio C. Spagnoli, University of Basel, Switzerland) was studied by immunoperoxidase staining performed in a traditional way on formalin-fixed, paraffin-embedded tissue samples. Microwave treatment in 10mM/l citric acid for 10 min was carried out, or Dako Target Retrieval Solution (S1699) (DakoCytomation, Glostrup, Denmark) was used, to enhance the antibody penetration. The slides were thereafter incubated with antibody and the staining was visualized using the NovoLink (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) polymer detection systems. Amplification of the primary antibody reaction was accomplished by incubating the sections with biotinylated secondary antibody (Vector Elite ABC Kit; Vector Laboratories Inc., Burlingame, CA, USA) or TSA Indirect (Pyramide Signal Amplification Kit, Perkin-Elmer LAS Inc., NEL700).

In study II, the ratio of MAGE-A positive tubules indicating spermatogonia in each sample was semiquantitatively assessed as a percentage of positive tubules/sample. At least 200 tubules were counted for each sample. Stainings of gonocyte markers octamer-binding transcription factor 3/4 (OCT 3/4) (Santa Cruz Biotechnology Inc, CA, USA), placental-like alkaline phosphatase (PLAP, Dako), and Sertoli and Leydig cell marker inhibin alpha-subunit (Serotec, Oxford, UK), were performed in the Labvision immunostainer (Labvision, CA, USA) and visualized utilizing the Dako EnVision polymer detection system (K5007, Dako).

In study IV, the primary antibody used for the MUL fibrothecomas was anti-inhibin- α (MCA9515; Serotec, Oxford, UK; 1:80). For TRIM37 staining of normal ovary samples, an antigen affinity-purified fraction of rabbit antiserum (60 μ g/ μ l) raised against a synthetic peptide (FPDGEQIGPEDLSFNTDENSGR) corresponding to the C terminus of the TRIM37 protein was used. The TRIM37 staining was carried out using Vectastain Elite kit (Vector Laboratories) according to the manufacturer's protocol. Immunostaining of inhibin- α was carried out in a Dako TechMate 500 automated staining machine.

4.7 Analysis of Sporadic Fibrothecomas (IV)

For the *TRIM37* mutation analysis of sporadic fibrothecomas, 15 freshly frozen samples of these tumors were obtained from the archives of the Department of Obstetrics and Gynecology. Messenger RNA was extracted from the specimens using the Oligotex mRNA midi kit (Qiagen, Hilden, Germany). Next, cDNA was synthesized using M-MLV reverse transcriptase (Promega, Madison, WI, USA) and random hexamers (Promega) according to the manufacturer's protocols. Seven overlapping fragments covering the whole open-reading frame of *TRIM37* were subsequently amplified by polymerase chain reaction (PCR) using primers and conditions described in detail in Avela et al. 2000. The resulting PCR products were assessed on 1% ethidium bromide-stained agarose gels, purified (PCR purification kit; Qiagen), and sequenced using the ABI PRISM Dye Terminator, the ABI PRISM dRhodamine cycle sequencing Kit (PerkinElmer, Foster City, CA, USA) or Big Dye Terminator (Applied Biosystems, Foster City, CA, USA) and run on an ABI Prism 310 Genetic Analyzer (Perkin Elmer). Both strands were sequenced and the acquired sequence information was edited, aligned and compared to the *TRIM37* cDNA sequence (GenBank NM 015294.1) using Sequencher 3.1 (Genes Codes Corporation). The 15 fibrothecoma specimens were further analyzed by TRIM37 staining as described in chapter 4.6. In addition, loss of heterozygosity (LOH) analysis and methylation analysis of the fibrothecoma specimens were carried out, as described in detail in the original publication IV.

4.8 Ethics

The Institutional Ethics Review Board at the University of Helsinki approved the study, and the National Authority for Medico-Legal Affairs in Finland (TEO) approved the use of clinical and autopsy material for research intentions. All patients or their parents or guardians provided informed consent. In addition, permission to use patient photographs was obtained.

5. RESULTS

Sexual maturation, fertility, and tumor predisposition were analyzed in the national cohort of the Finnish MUL patients by clinical, hormonal, histological, and immunohistochemical methods. The results indicate that MUL is a monogenic disorder causing human infertility and failure of sexual maturation as well as a high risk of both benign and malignant tumors.

5.1 Sexual Maturation and Fertility in MUL Females

5.1.1 Gonadal Function in Prepubertal and Pubertal MUL Girls (I, Unpublished Data)

MUL girls overall presented relatively normal genital anatomy, although the labia were often edematous. Serum gonadotropins were normal and the onset of puberty occurred spontaneously. Breast development was poor and remained incomplete, while none of the girls reached Tanner stage M5. The pubertal growth acceleration commenced with the onset of breast development, but was weak and often not possible to discern. Pubic hair generally appeared before the onset of breast development and was adequate. Median age at menarche was 14.7 years but showed wide individual variation (range 9.8-17.8 years). Of the girls, 45% never attained regular periods. Five years after menarche, only three patients (14%) menstruated regularly.

5.1.2 Gonadal Function and Fertility in Adult MUL Women (I, Unpublished Data)

MUL women displayed hypoplastic breasts and pubic hair was fairly normally distributed (Figure 13). The labia were often edematous and the uteri were small. The first signs of ovarian failure appeared approximately 1.8 years after menarche as a menstrual irregularity, ranging from oligomenorrhea to long periods of amenorrhea with episodes of menometrorrhagia. Hormone replacement therapy (HRT) or oral contraception (OC) had been initiated for the majority of women 1-10 years after menarche. The age for menopausal amenorrhea could be estimated only for four patients without HRT; it was between 18 and 38 years. None of the women had been pregnant, but an ovulatory cycle had been confirmed in four patients by repeated ultrasound follicle examination (in three) and/or luteal phase progesterone measurement (in two). Half of the adult women in the cohort were living in a settled relationship and the majority suffered from involuntary infertility. The women reported satisfactory sexual desire. Serum FSH measurements revealed elevated concentrations in 10/15 (67%) of the women, indicating ovarian failure. LH concentrations were elevated in 8/15 (53%) of the women. Estrogen levels were in the low/normal range. Inhibin B levels were elevated in three patients, two of which were

concomitantly diagnosed with an ovarian tumor, fibrothecoma (Table 5). The third patient with elevated inhibin B levels was diagnosed with a fibrothecoma three years after the completion of this study.

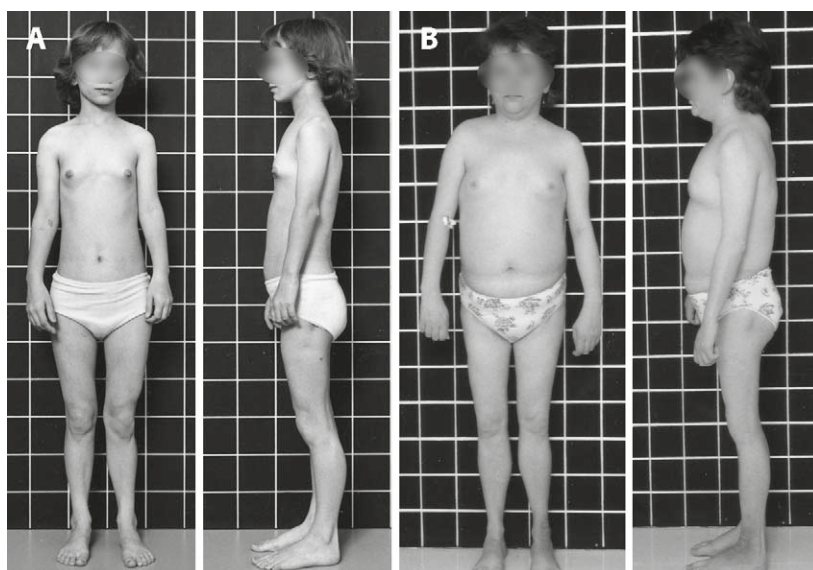


Figure 13. A. An 18-year-old MUL woman. B. A 31-year-old MUL woman. Note the hypoplastic breasts and the difference in body composition with increasing age.

Table 5. Hormonal parameters of the 15 females in the clinical study

Patient	Age (years)	FSH (IU/l)	LH (IU/l)	E2 (nmol/l)	SHBG (nmol/l)	Inhibin B (ng/l)	T (nmol/l)
Normal range		<10 IU/l**	<10 IU/l***	0.11-0.44 nmol/l	19-101 nmol/l	5.0-200 ng/l	0.9-2.8 nmol/l
1	15	19.7	10.8	0.03	23	18	0.5
2*	16	6.4	30.2	0.08	36	241	0.8
3*	19	15.9	5.1	0.19	30	213	1.0
4	20	8.5	2.3	0.05	56	31	1.3
5	22	7.7	7.8	0.20	115	34	1.6
6	23	127.0	95.9	0.06	28	24	1.2
7	24	11.8	13.6	0.42	20	97	1.0
8	28	27.7	29.4	0.15	36	47	1.1
9	30	15.3	9.5	0.29	84	33	1.7
10	31	49.7	26.1	0.09	40	<15.6	3.3
11	33	9.9	4.8	0.50	57	65	0.5
12	35	53.2	30.7	0.05	72	17	1.7
13	35	89.5	62.2	0.19	36	39	0.9
14	39	21.2	7.4	0.08	79	18	3.9
15*	48	1.2	7.2	0.03	52	383	1.0

* Patients 2, 3 and 15 were later diagnosed with fibrothecoma, explaining the markedly elevated inhibin B levels and modest FSH levels, ** follicular phase: 1.0-10 IU/l, luteal phase: 1.0-8.0 IU/l, *** follicular phase: 2.0-10 IU/l, luteal phase: 1.0-13 IU/l.

Pelvic US revealed hypoplastic ovaries with no follicles in 11/15 (73%) of the women. Three patients had small ovaries with only few antral follicles, and only one subject (compound heterozygote) had ovaries with normal morphology. Six patients had previously undergone surgery for fibrothecomas, and six additional tumors were diagnosed by pelvic US during this study. Altogether, ovarian fibrothecomas were diagnosed in 12/22 women, thus presenting a risk of fibrothecoma of 55%. All except for one patient presented with oligomenorrhea or amenorrhea before tumor diagnosis (Figure 14).

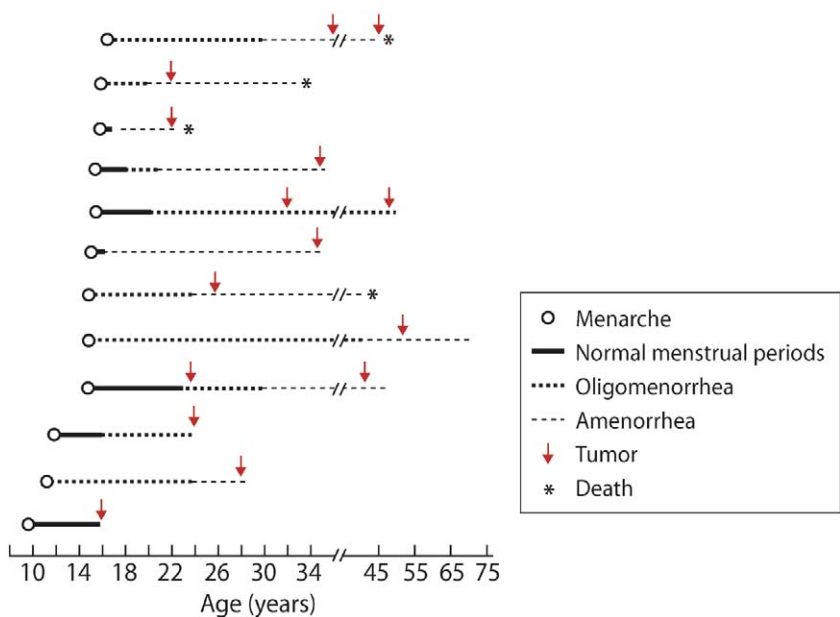


Figure 14. Menstrual history and age at tumor diagnosis in the 12 MUL females with fibrothecomas. Adapted from The New England Journal of Medicine (1).

5.2 Sexual Maturation and Fertility in MUL Males

5.2.1 Gonadal Function in Prepubertal and Pubertal MUL Boys (II)

Genital anatomy appeared to be normal in MUL boys, although the testes were already somewhat small in childhood (testicular volume 1-2 ml). Two boys (2/28, 7.1%) demonstrated surgically corrected unilateral cryptorchidism, and one had congenital cryptorchidism with spontaneous descent, before one year of age. No one presented with hypospadias or micropenis. In childhood and prepuberty, serum levels of FSH, LH, testosterone, and inhibin B were all within the normal range.

Similar to MUL girls, the onset of puberty in boys showed wide individual variation but occurred spontaneously at a median age of 12.6 years (range 11.1-15.0). The progression of puberty was fairly normal but incomplete. The majority (72%) of boys never reached Tanner stage P5G5, while testicular volume failed to reach adult measures and/or pubertal hair remained at Tanner stage 4. No patients had signs of gynecomastia. FSH and, to some extent, LH gradually increased, from the beginning of midpuberty, to hypergonadotropic levels. After an initial normal adolescent increase, testosterone concentrations leveled off and subsequently remained within the low-normal range throughout puberty. As puberty progressed, inhibin B showed a clear suppression to low levels concomitantly with the FSH increase (Figure 15).

5.2.2 Gonadal Function and Fertility in MUL Men (II)

The median testicular volume in the 20 MUL adults was 8.7 ml (range 3.5-18.3 ml), while the median testicular volume in healthy Finnish men is 21 ml (range 15-28) (Jørgensen et al. 2011). After puberty, the size of the testes typically decreased with time and they became softer. All adult men showed signs of hypergonadotropic hypogonadism characterized by elevated FSH and sometimes also LH levels, low-normal testosterone levels and low inhibin levels (Figure 15). In males over 26 years of age, FSH was increased in 85%, while LH was elevated in 38%. The two men with FSH concentrations in the normal range, did however exhibit exaggerated responses to GnRH. Most of the adults (12/19) were either married or living in a settled partnership. The majority (10/12, 83%) reported satisfactory sexual desire and function, but two men (2/12, 17%) complained of suppressed libido.

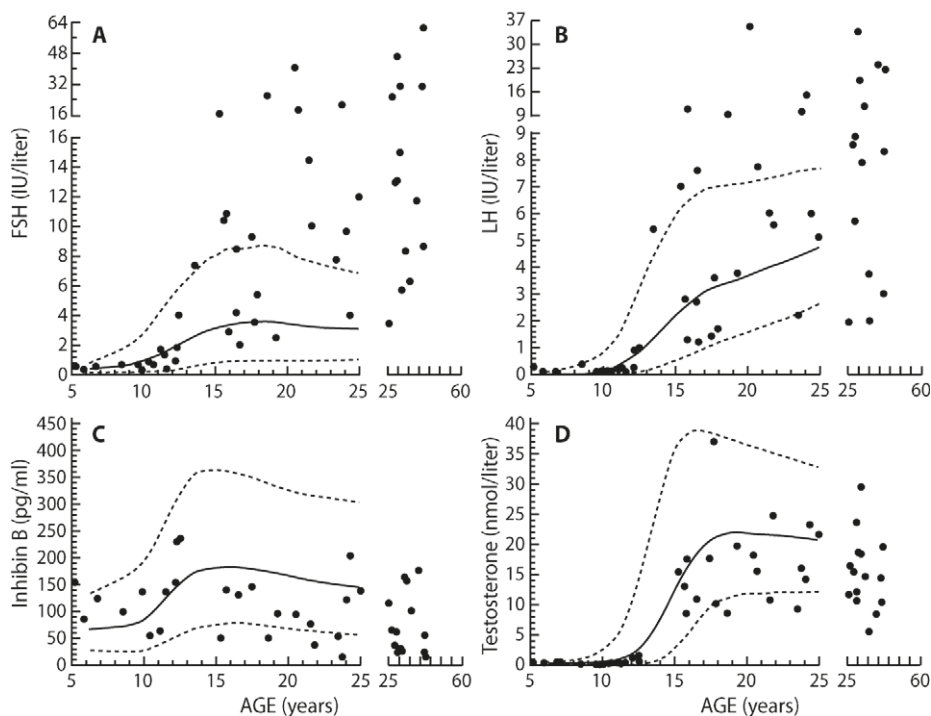


Figure 15. FSH, LH, inhibin B, and testosterone levels in MUL males, plotted as a function of age. Upper and lower limits of normal indicated by dashed lines. Adapted from the *Journal of Clinical Endocrinology and Metabolism* (II).

Although 20 men were over 16 years-of-age in the cohort, 17 of these provided a semen sample. All samples were pathological (Table 6). They showed either severe oligoasthenozoospermia ($n=13$) or azoospermia ($n=4$). The median ejaculate volume was 2.0 ml (range 0.3-5 ml), being subnormal (<1.5 ml according to WHO) in 35% of the patients. Median sperm concentration was 0.007×10^6 /ml. Only one patient had spermatozoa with rapid progression (WHO class a), the rest had significantly reduced sperm motility. Due to the low sperm density, morphological analyses could be carried out only in one sample, which showed gross teratozoospermia (Table 6).

No man had a history of spontaneous fertility. Four men had undergone infertility treatment and in one case the treatment (intracytoplasmic sperm injection, ICSI, with fresh ejaculated sperm) was successful resulting in the delivery of a healthy child.

Table 6. Comparison between median values for semen variables in 17 MUL men and WHO 2010 reference limits for semen characteristics

Parameter	MUL (range)	WHO
Semen volume (ml)	2 (0.3-5)	>1.5
Total sperm number (10^6 per ejaculate)	0.022 (0-3.6)	>39
Sperm concentration (10^6 per ml)	0.007 (0-2.4)	>15
Progressive motility (%)	0 (0-10)	>32
Total progressive motility (%)	0 (0-48)	>40

5.2.3 Histopathology of MUL Testes (II)

The histological structure of the MUL testes was evaluated in four children and four adults. The testes of the three younger MUL children (between 2 – 4.9 years of age) showed large Sertoli-cell-only (SCO) areas with only a paucity of seminiferous tubules containing any spermatogonia (Figure 16). In contrast, the number of Sertoli and Leydig cells appeared normal. Also the one pubertal sample examined, from a patient aged 14 years, showed large SCO areas, although more seminiferous tubules contained spermatogonia than the samples of the young children. In the four adult samples (aged between 24-38 years), the testes were characterized by prominently thin germinal epithelia. Advanced spermatogenesis, as indicated by the presence of spermatids, was, however, seen in few tubules (Figure 16). All four adult samples showed Leydig cell hyperplasia. One patient displayed a rare sex cord-stromal tumor, thecoma.

Immunohistochemical staining of all 12 testicular samples was performed in order to characterize the testicular degeneration process. Gonocyte markers, OCT3/4 and PLAP, were not expressed in any of the samples, however spermatogonium marker MAGE-A was expressed in all spermatogonia in both MUL and control samples. All MUL samples exhibited a patchy MAGE-A appearance in agreement with the focal degeneration process. The mean percentage of MAGE-A positive seminiferous tubules was 53% in MUL patients and 88% in controls. Inhibin-alpha showed strong staining in Sertoli and Leydig cells in both MUL and control subjects. In MUL samples, inhibin-alpha staining showed marked Leydig cell hyperplasia, almost resembling adenomatous lesions.

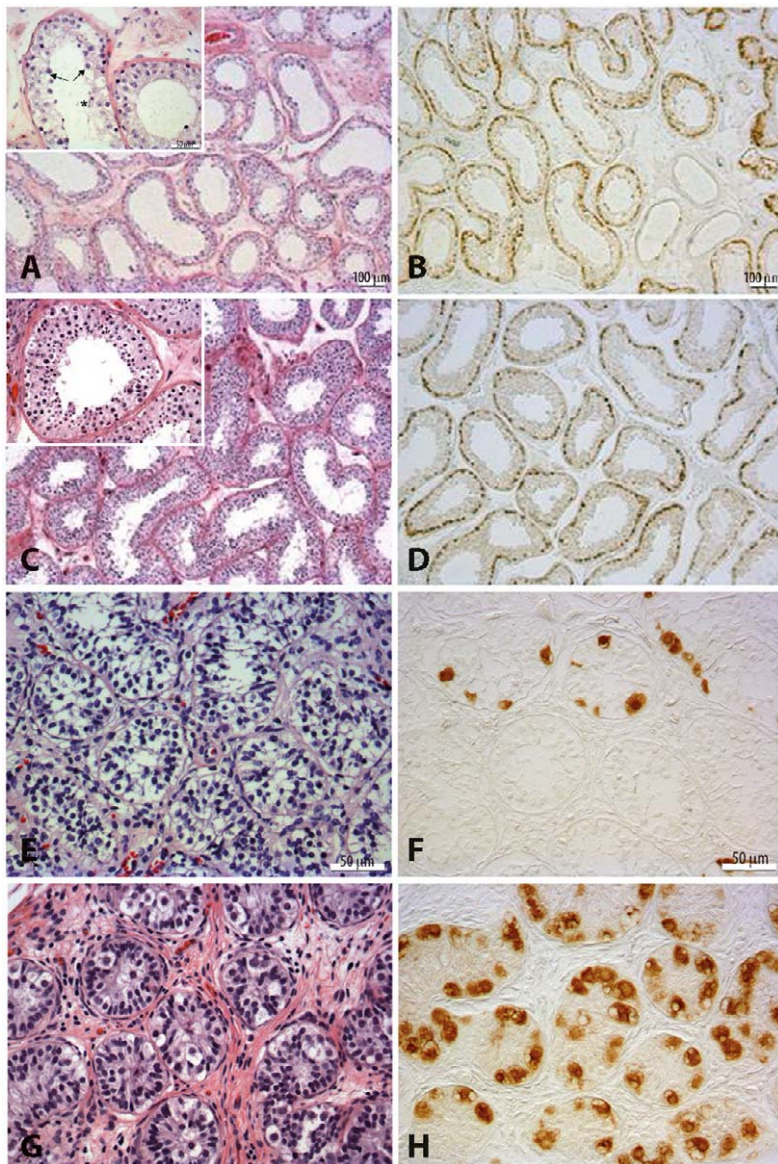


Figure 16. Typical testicular histology of MUL patients in comparison with normal controls. **A)** Hematoxylin and eosin (HE) staining of an adult MUL testis. Note the thin seminiferous tubules. Only a few pachytene spermatocytes (arrows) and spermatids (asterisk) can be seen in the magnification in the insert picture. **B)** MAGE-A staining of the same MUL patient. Note three Sertoli cell-only tubules in the lower right corner. **C)** HE staining of normal adult control, note the thick seminiferous epithelia. **D)** MAGE-A staining of the same control. **E)** HE staining of the testis in a MUL child. **F)** MAGE-A staining of the same MUL child; note the very few spermatogonia and the predominance of Sertoli cell-only tubules. **G)** HE staining of a normal control child. **H)** MAGE-A staining of the same control with abundance of spermatogonia. Partly adapted from the Journal of Clinical Endocrinology and Metabolism (II).

5.3 Tumor Formation and Organogenesis in MUL (III, IV)

5.3.1 Tumors

MUL patients presented with a high frequency of benign tumors comprising mostly cystic and benign adenomatous lesions. Altogether 232 tumorous lesions were detected in 76 patients and 17 autopsy samples (Figure 17). The most consistent tumorous lesion was found in the liver, where lesions resembling hemangiomas were found in 49% (35/72) of patients by US. The lesions were 5-60 mm in size and frequently enlarged with time. In one-third of the patients, however, the lesions fluctuated in size during follow-up and temporarily vanished in five patients. In all 17 deceased patients (between 0.7 and 48 years of age), the liver was enlarged and congestive at autopsy. In 16 of these (94%), multiple macroscopic lesions of 5-70 mm resembling hemangiomas were found. Histological examinations of specimens obtained from the 17 autopsies and from 20 liver biopsies (patients between 2.4 to 40 years of age) disclosed peliosis, i.e. marked sinusoidal dilatation with a disorganized pattern in relation to central veins and portal tracts. In these lesions, the lobular architecture was focally disturbed and the central veins in these areas were dilated and had unusually thick walls. Hepatocytes displayed a normal, or slightly enlarged, appearance with variable amounts of micro- and macrovesicular lipid droplets. The portal tracts and biliary tracts appeared normal, but the degree of fibrosis increased with age. No lesions showed evidence of malignancy. Fatty liver was continually noted in both the liver biopsies (14/20) as well as in the autopsy specimens (13/17).

Half (52%) of the patients had renal cortical cysts, 26% had pancreatic cysts, 20% had thyroid cysts, 15% had cysts in the central nervous system (CNS), and 11% had cysts in the reproductive organs. Bilateral nodular cortical hyperplasia of the adrenal gland was found in 87% of the histological samples analyzed (13/15). In the pancreas, hyperplastic islets, as seen in focal endocrine hyperplasia, were evident in all samples analyzed (13/13). In addition, 25% of the patients (n=22) had fibrous dysplasia of long bones, four had nodular thyroid goitre, two presented with renal angiomyolipomas, one had a renal hamartoma containing ovarian tissue, one had a pancreatic serous cystadenoma, and one infant presented with a small medullar pheochromocytoma. In addition, six adrenal cortical adenomas and two thyroid adenomas were discovered. In the female patients, the most frequently found tumors were benign ovarian fibrothecomas, which were encountered in more than half (12/22) of the post-pubertal females.

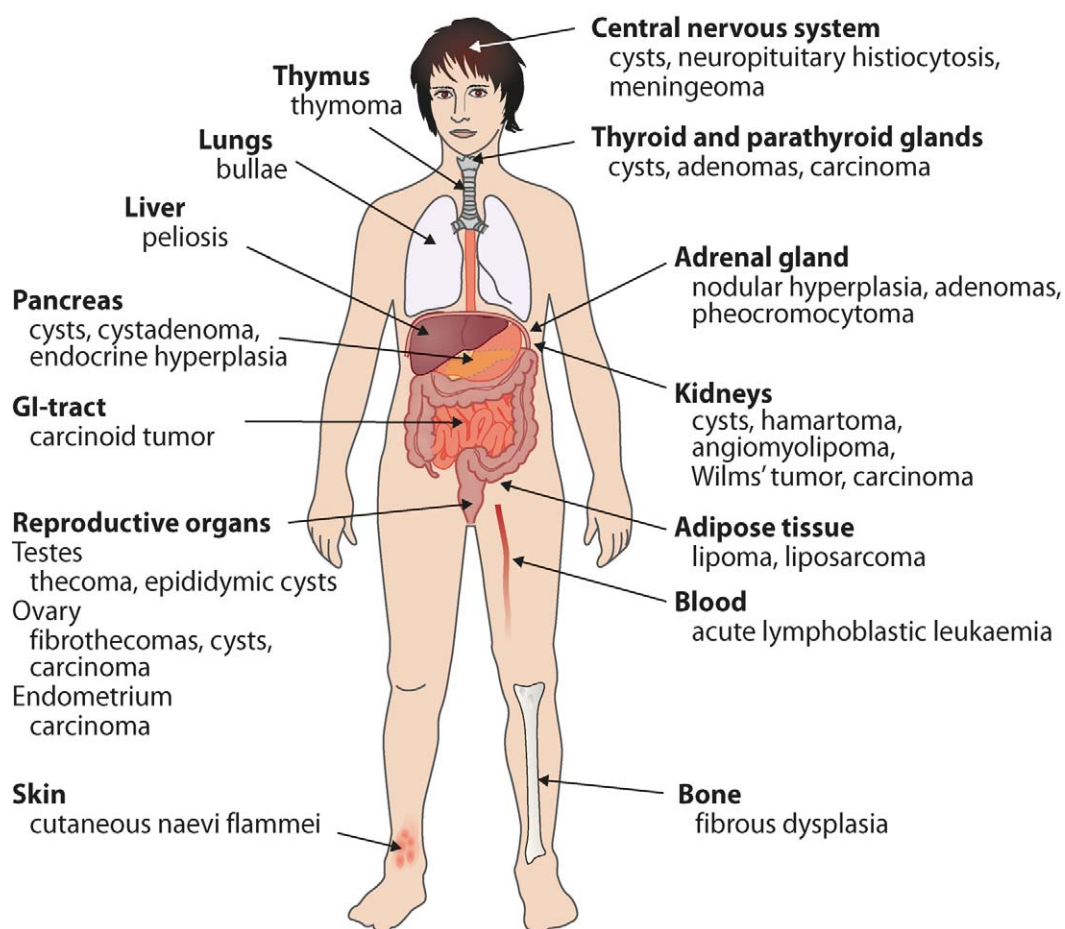


Figure 17. Distribution of tumors in MUL.

Malignant tumors were detected in 15% of the patients (15 tumors in 13 patients), most commonly in the kidneys. Wilms' tumor was diagnosed in five (6%) of the patients at a mean age of 2.5 years, and two subjects carried a renal papillary carcinoma. In addition, two patients presented with thyroid papillary carcinoma, one with a medullar thyroid carcinoma in combination with squamous-cell metaplasia, one with a gastrointestinal carcinoid tumor, one with a neuropituitary Langerhans cell histiocytosis, and one with acute lymphoblastic leukemia. Two gynecological malignancies were discovered; one ovarian carcinoma and one endometrial adenocarcinoma.

5.3.2 Organogenesis in MUL

Signs of disturbed organ development were apparent in many internal organs. In the adrenal gland, the general morphology was clearly disturbed; cortical and medullary structures intermingled and often the adrenal parenchyma penetrated through the capsule into the surrounding renal tissue or fat. In addition, many histological specimens showed a fetal-type cortical architecture. One female patient had a focus of ovarian tissue in her kidney. All histological specimens displayed an abnormally folded pancreatic gland with lipid deposits within and surrounding the gland. The spleen was congestive in all autopsy samples, containing lymphocytes, but lacking lymph follicles in the white pulpa. Correspondingly, a serious lymphatic depletion in the lymph nodes was evident.

5.3.3 Immunohistochemistry of Tumor Samples (III)

The histological samples of the internal organs often showed strongly dilated and folded blood vessels with abnormally thick walls. Especially in the hilar region of the lymph nodes, the blood vessels were markedly large and folded. Immunohistochemistry revealed strong positive staining for the endothelial cell markers CD31 and CD34 as well as for the myocyte marker α -SMA. In contrast, staining for proliferation marker MIB-1, p53 and the marker for PEComa, HMB-45, were negative.

The peliotic lesions in the liver also showed strong positive staining of endothelial cell markers, CD31 and especially CD34, as well as of the myocyte marker α -SMA (Figure 18). The staining pattern clearly differed from control livers, where the staining of all markers was restricted to portal areas. Mitotic activity in the peliotic lesions was scarce and the expression of MIB-1 was also weak. The expression of HMB-45 was negative.

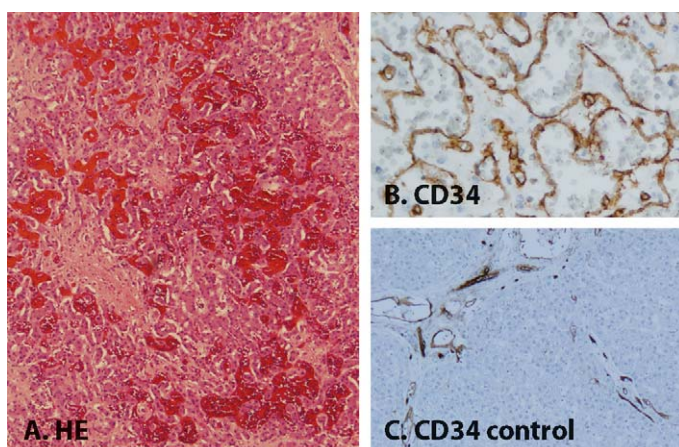


Figure 18. Typical liver pathology in MUL. A) A peliotic lesion with strong sinusoidal dilatation. B) Strong staining of the endothelial marker CD34 in comparison with a healthy control (C). Partly adapted from the Journal of Pathology (III).

5.3.4 Ovarian Tumors in MUL (IV)

Ovarian sex cord-stromal tumors were found in 12 of the 22 (55%) women in the cohort. Seven of the tumors were diagnosed as thecomas, four as fibromas, and one as a cellular fibroma (Table 7) (Figure 19). Mean age at first tumor diagnosis was 29 years (range 16-52 years). The size of the tumors varied from 1 to 15 cm with a mean diameter of 7 cm. Five of the tumors (42%) were multifocal and in half of the cases the tumors were bilateral. The smaller tumors had clear margins, whereas the ovarian morphology was usually disturbed in larger tumors. Two patients developed recurrent fibrothecomas, 17 and 18 years after the primary surgery. Of the 12 fibrothecomas, 9 (75%) showed inhibin-alpha positivity in immunohistochemical staining.

Table 7. Fibrothecomas in MUL females

No.	Age *	Tumor	Diameter (cm)	Bilateral	Multifocal	Recurrence
1	16	Thecoma	14			
2	22	Thecoma	1			
3	22	Thecoma	5	+	+	
4	24	Thecoma	8			
5	24	Fibroma+AF	7	+	+	+
6	26	Fibroma	8	+	+	
7	28	Fibroma	3	+		
8	32	Fibroma+AF	6	+	+	+
9	35	Thecoma	5			
10	35	Thecoma	1		+	
11	36	Thecoma	15			+
12	52	CF	8	+		

* Age at tumor diagnosis. CF, cellular fibroma, AF, adenofibroma. Adapted from Modern Pathology (IV)

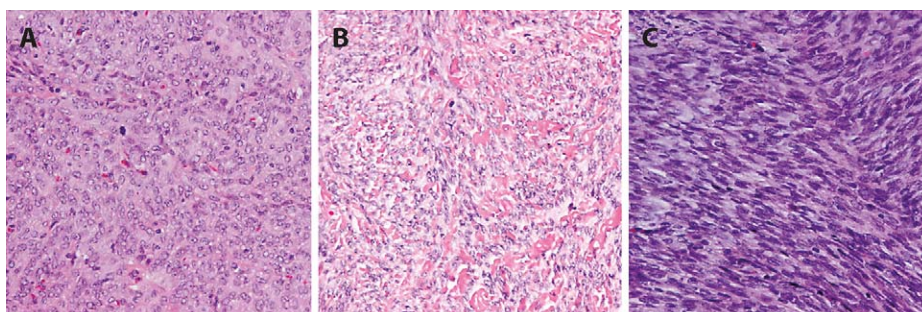


Figure 19. A) MUL-associated thecoma B) MUL-associated fibroma and C) MUL-associated cellular fibroma. Partly adapted from Modern Pathology (IV).

5.3.5 Sporadic Fibrothecomas (IV)

In order to analyze the potential role of *TRIM37* mutations in the etiology of sporadic fibrothecomas, *TRIM37* cDNA was sequenced from 15 sporadic tumor specimens utilizing seven overlapping amplicons. No mutations in *TRIM37* were found. In one tumor, a base-pair change from C→T at nucleotide 398 was discovered, resulting in a probable non-significant substitution of valine for alanine. In the expression analyses 80% of the sporadic fibrothecomas showed either completely negative (48%) or weak (32%) staining for TRIM37 protein, and only 20% of the sporadic tumors exhibited immunopositivity that was comparable to normal thecal cells (Figure 20). To further analyze the possible genetic and epigenetic events leading to the reduced expression of TRIM37 in sporadic fibrothecomas, LOH and CpG island promoter hypermethylation in these tumors were analyzed. Six percent of the sporadic fibrothecomas examined showed LOH at the *TRIM37* locus. Methylation of the TRIM37 promoter area was present in 48% of the sporadic fibrothecomas examined, suggesting that this could also be one mechanism in the TRIM37 inactivation.

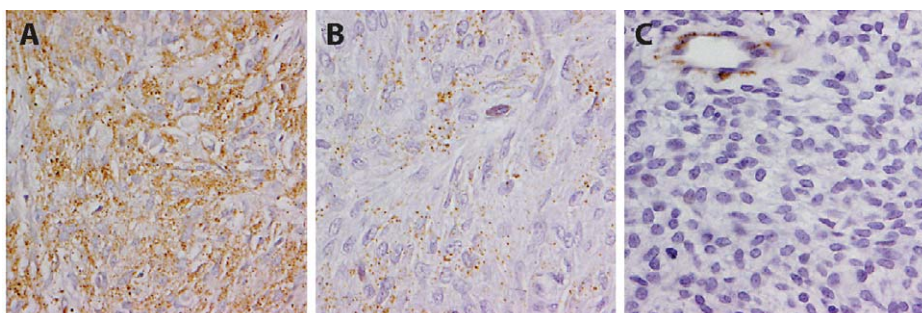


Figure 20. A) Sporadic fibrothecoma highly expressing TRIM37, B) sporadic fibrothecoma with low TRIM37 expression and C) sporadic fibrothecoma completely negative for TRIM37. Note positive staining of capillary wall cells. Original magnification x 200. Partly adapted from Modern Pathology (IV).

6. DISCUSSION

The sexual maturation, gonadal function and tumor predisposition of a well-characterized population of MUL children and adults with a defect in a single gene *TRIM37* were evaluated here. The results show that all MUL adults develop hypogonadism due to a primary gonadal defect and they are either infertile or severely subfertile. Furthermore, these patients experience disturbances in organogenesis and are at a high risk of developing both benign and malignant tumors in various organs.

6.1 Sexual Maturation and Gonadal Function in MUL Women

MUL women experience spontaneous, albeit often late, puberty with incomplete breast development, and early irregularity of menstrual periods with subsequent ovarian failure and infertility. The clinical spectrum varied from a normal or even early menarche with some years of regular periods to a very late menarche with apparent direct transition into severe oligomenorrhea followed by amenorrhea, indicating the development of POF.

The clinical spectrum also varies in non-syndromic POF, and there is no menstrual history that is characteristic for the development of POF (Nelson 2009). Most often, the disorder develops after a normal puberty and regular menses, but in about 10% of cases, POF may present as primary amenorrhea (Rebar and Connolly 1990). Only 3/22 of MUL women menstruated regularly after 5 years from menarche. Transition to amenorrhea followed menarche, after a time span of between a few months to 13 years, in the absence of hormonal treatment. The interval could not be assessed more accurately, as menstrual disturbances progressed intermittently, hormone assays were not performed regularly, and many of the patients received contraceptive pills, cyclic progestin treatment, or HRT to control bleedings.

In the majority of POF cases, the underlying cause remains unknown. The detection of several causal genetic defects in humans, experimental models, and the recurrent familial forms (in about 10-15% of cases), all point towards the fact that POF has a strong genetic component (Persani et al. 2010, van Kasteren et al. 1999). However, POF is a heterogeneous disease and even within the same family, the expressivity can vary. Thus, in addition to monogenic forms, POF is probably a multifactorial disorder in many cases, resulting from the contribution of several predisposing alleles (Persani et al. 2010, Shelling 2010). Ovarian failure can result from initially deficient follicle number, abnormally rapid follicular loss, or resistance to gonadotropins, and the primary defect can lie in the oocytes or the surrounding follicle cells. Normally, the follicular atresia already commences in fetal life and the age at ovarian failure is deter-

mined by the size of the oocyte reserve and the rate of its depletion. Whether the oocyte reserve in MUL is deficient at the outset, or if the rate of follicular atresia is exaggerated, remains to be elucidated.

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein belonging to the transforming growth factor- β family (Cate et al. 1986), which in recent years has emerged as a novel measure of the ovarian reserve. In females, granulosa cells of the primary, pre-antral, and small antral follicles produce AMH. The number of small antral follicles is associated with the extent of the primordial follicle pool (Kevenaar et al. 2006), and as the number of antral follicles decreases with age, serum AMH levels decline, becoming undetectable in menopause (Van Rooij et al. 2004, 2005). Consequently, serum AMH levels are also considered to be a marker for ovarian aging (Broekmans et al. 2008, Broer et al. 2011). AMH is also a good predictor of ovarian damage in POF, as AMH levels are mostly either significantly diminished or undetectable in this condition (La Marca et al. 2006, Meduri et al. 2007, Kallio et al. 2012). Accordingly, in girls with Turner syndrome (TS), serum AMH levels show promising results in assessing ovarian reserve and thus potential fertility (Borgström et al. 2009, Purushothaman 2010). In future assessment of the ovarian reserve in MUL girls and women, determination of AMH levels will prove valuable.

6.2 Fertility in MUL Women

None of the MUL women reported a history of spontaneous pregnancies and all women eventually developed POF. In recent years, there has been some debate about whether the term “premature ovarian failure” should be substituted in favor of the terms “premature ovarian insufficiency” or “premature ovarian dysfunction”, which reflect the possibly reversible nature of the condition and have less negative connotations than the word “failure”. However, so far no consensus has been reached and in the case of MUL, POF seems like an accurate term to use, since ovarian recovery in MUL has never been documented and is extremely unlikely.

Infertility is an important issue for most women with POF. Treatments with clomiphene, gonadotropins, GnRH agonists, and immunosuppressants have not been effective in restoring fertility (Bidet et al. 2008), and the only reliable treatment option so far is the use of donor eggs and IVF (Shelling 2010). Embryo cryopreservation is already an established method for fertility-preservation. Cryopreservation of ovarian tissue or oocytes is still considered experimental, but may be readily available in the future (Picton 2008, Rodriguez-Wallberg and Oktay 2012). Women with POF probably have follicles of lower quality, however, meaning that the follicles should be collected well before the ovarian failure actually develops, which requires that the women would be aware of an impending POF.

It is very uncertain how the body and the uterus of a MUL woman would adapt to a possible pregnancy. Pregnancy is associated with major hemodynamic changes and thromboembolic risks. Thus, the potential impact that a pregnancy may have on the heart disease associated with MUL is a significant issue to be taken into account. So far, the risks have been considered too large to promote assisted reproductive therapies for MUL women.

POF and polycystic ovarian syndrome (PCOS) represent two important forms of female infertility. POF usually results from an early depletion or dysfunction of the oocyte population, whereas PCOS is characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovaries, with an accumulation of small antral follicles (Goodarzi et al. 2011). In a large proportion of infertile females, however, the cause still remains a mystery.

6.3 Sexual Maturation and Gonadal Function in MUL Males

A reduction in the number of seminiferous tubules containing germ cells was already evident in young and prepubertal MUL boys, but Sertoli and Leydig cells appeared to be normal. Serum levels of FSH, LH, testosterone, and inhibin B were within normal ranges. The onset of puberty, as assessed by the appearance of pubic hair and initial testicular enlargement, was also within normal limits for healthy Finnish boys. Similar to MUL girls, however, a trend toward late puberty onset was apparent. Given that MUL boys already presented with smaller testes before puberty, a testicular volume of 2.0 ml, instead of the standard limit of 3.0 ml, was accepted here to indicate onset of puberty. FSH, LH, testosterone, and inhibin B levels increased adequately until midpuberty, after which the testicular growth and virilization proceeded slowly. Concurrently, inhibin B levels decreased and testosterone also leveled off, but FSH, and to a lesser extent LH, showed a progressive increase to hypergonadotropic levels.

All adult MUL men showed signs of hypergonadotropic hypogonadism. Their mean testicular volume was 8.7 ml, and none reached the normal median volume of 21 ml for healthy Finnish men (Jørgensen et al. 2011). Klinefelter syndrome (KS), although more severe, in many ways resembles the hypogonadal picture of MUL men. It is characterized by azoospermia with small firm testes of less than 4 ml in volume (Wikström and Dunkel 2011). Small and firm testes indicate that the germinal epithelium was never, or only transiently, established. In contrast, if testicular damage develops after spermatogenesis is established, the subsequent collapse of seminiferous tubules leaves the testis soft and flaccid. In MUL, the soft and shrinking testes indicate that although spermatogenesis is established, it is incomplete and evanescent, possibly due to an accelerated rate of germ cell apoptosis.

The degree of virilization varied and was usually incomplete, but appeared to be satisfactory for the patients. Examination of the testicular histology displayed varying degrees of degeneration. Sertoli cells appeared roughly normal, but Leydig cells characteristically formed hyperplastic areas. The histology of the MUL testes suggests an impairment of both the spermatogenic and the endocrine function. This impairment was confirmed by a reduction in testicular size during early adulthood, reflecting the progressive decrement of germ cells.

The increasing levels of LH observed after midpuberty plausibly reflect a compensatory feedback mechanism to sustain sufficient Leydig cell function and, subsequently, testosterone production. This is in line with the observed Leydig cell hyperplasia and quite satisfactory testosterone levels in adult MUL men. The testosterone level, below which symptoms of androgen insufficiency occur, is not known (Bhasin and Basaria 2011), although values below a threshold of 10.4 nmol/liter are associated with an increased likelihood of symptoms (Kelleher et al. 2004, Bhasin and Basaria 2011); only two adult MUL men displayed testosterone levels below this threshold. Accordingly, the majority of adult men in our cohort reported adequate sexual function; however, one third of the men had no present or past sexual relationships, which may indicate low sexual desire due to androgen deficiency.

In KS, androgen replacement treatment is offered to the majority of males from the peri-pubertal period in order to assure optimal development of sexual characteristics and to avoid sexual dysfunction (Nielsen et al. 1988). In MUL, with all its concurrent pathologies, the risk-benefit decision of testosterone treatment is more complicated. Testosterone treatment can potentially induce dyslipidemia, liver toxicity, and increased cardiovascular events, for example (Cunningham and Toma 2010), all of which could possibly worsen the clinical picture of MUL. Following this study, two MUL males were recommended testosterone substitution; one with low testosterone levels after unilateral orchiectomy, the other complaining about reduced sexual function and fatigue. In addition, exogenous testosterone treatment suppresses the HPG axis and thus spermatogenesis (McLachlan et al. 2002b, Cunningham and Toma 2011), which may also argue against a routine treatment of MUL males with androgens, especially those with a desire for fertility in the near future.

Whether the defect in the MUL testes is intrinsic to germ cells or is due to failure of Sertoli cells to support the germ cells still needs to be elucidated. In postpubertal male mice, TRIM37 is expressed in germ cells from type B spermatogonia to early round spermatids, while Sertoli cells, Leydig cells, early spermatogonia, and elongated spermatids are negative (Kallijärvi et al. 2006). This strongly implicates a primary germ cell defect. The expression of gonocyte markers, OCT 3/4 and PLAP, was negative in the testicular samples, indicating that gonocytes differentiate adequately to spermatogonia in MUL patients.

The testicular samples did contain spermatogonia, as evidenced by MAGE-A staining, but spermatocytes and especially spermatids were scant. This implies that germ cells have difficulty entering meiosis and that germ cell differentiation is, at least in part, arrested at the spermatogonium stage. One feasible explanation could be an accelerated rate of germ cell apoptosis, the reasons for which still need to be elucidated. Interestingly, the samples of the autopsied children displayed the most severe histology, probably reflecting the severity of the disease, whereas all children had died at young age of MUL complications. The pathogenic mechanisms underlying the process of testicular degeneration in MUL remain unknown, but the mechanisms leading to the loss of germ cells evidently start early in life, possibly already during fetal life, and the fertility potential is presumably reduced even before puberty.

6.4 Fertility in MUL Men

All MUL men presented with severe oligoasthenozoospermia or azoospermia. The sperm concentrations ranged from 0-2.4 million/ml, whereas the recent WHO reference level is over 15 million/ml (World Health Organization 2010). *In vitro* fertilization (IVF) by ICSI, using either ejaculated sperm or testicular sperm extraction (TESE), gives hope for biological paternity to MUL men. To date, four MUL men have gone through infertility treatment including ICSI, one of them also with TESE, but only one successful pregnancy has been conceived thus far, using ICSI with fresh ejaculated sperm. TESE also seems like a promising method for MUL men, since in Klinefelter patients, it has proven successful even with no sperm in ejaculates (Schiff et al. 2005, Vernaev et al. 2004). Thus, one important issue to take into consideration is the possibility of cryopreserving semen samples from MUL boys during early puberty, postulating that the testicular degeneration process in MUL is progressive.

The molecular mechanisms leading to spermatogenic damage in cases of genetic infertility are still unknown. Chromosomal aberrations, such as Klinefelter syndrome and Y chromosome microdeletions, account for roughly 5% of infertility in males (Ferlin et al. 2007). Only few monogenic mutations causing male infertility have been recognized in humans. Recently, Bashamboo et al. showed that missense mutations in *NR5A1*, encoding for a key transcriptional regulator of genes involved in the HPG-axis (Luo et al. 1994), account for 4% of unexplained spermatogenic failure in otherwise healthy males (Bashamboo et al. 2010). *NR5A1* plays an important role in gonadal development, as it regulates *SOX9* (Sry-box 9) and *AMH* during testis determination and differentiation, and regulates the expression of many factors involved in steroid hormone biosynthesis and cholesterol mobilization, including *HMG-CoA* synthase (Lin and Achermann 2008, Sekido and Lovell-Badge 2008). This finding is highly interesting, since mutations in *NR5A1* are also associated with POF (Lourengo et al. 2009). Thus, both *TRIM37* and *NR5A1* represent single-gene defects capable of inducing both testicular as well as ovarian failure.

Based on our results, it seems clear that all MUL patients will ultimately develop some level of gonadal failure, and therefore the question of whether gonadal failure should be added to the list of major diagnostic signs in MUL is justified. Many gaps remain in our understanding of the etiology of POF and testicular failure. Perception of the basic pathophysiologic mechanisms in these conditions, which can be aided by the study of monogenic models, such as MUL, provides an opportunity for early intervention, and is crucial for the development of new therapies for these patients. Consequently, MUL can be regarded as a model disease for infertility, and interestingly, MUL is one of few syndromic forms of infertility where both females and males are affected.

6.5 SGA and Gonadal Function

Children with impaired fetal growth are well known to be at a higher risk for perinatal morbidity and mortality and for a number of chronic diseases later in life, such as hypertension, insulin resistance, type 2 diabetes, and cardiovascular disease (Hales and Barker 1992, 2001, Barker 1995).

Growth retardation can result in defective organ systems that may function suboptimally later in life. Each organ probably has its own critical time window during gestation in which adequate development can be affected (Barker 1992). In pregnancies, which are complicated by fetal hypoxemia and growth retardation, the fetal kidneys are affected by reduced growth, development and functional capacity (Kurjak et al. 1981, Deutinger et al. 1987, De Bruin et al. 1998). Interestingly, the developing kidneys and reproductive organs and their respective vascular systems are strongly linked in fetal life. As a consequence of this near anatomical relation, it is very likely that during placental insufficiency, not only the kidneys, but also at least the ovaries may suffer from a reduced blood flow, which could result in an unfavorable environment for the developing follicles and thus result in a diminished follicle population at birth (De Bruin et al. 1998).

There are conflicting data about the relationship between SGA and gonadal function in both women and men. In infant and adolescent girls, prenatal growth failure has been associated with a decreased number of primordial follicles (de Bruin et al. 1998), elevated serum FSH concentrations, hyperandrogenism, reduced ovulation rates, as well as small uterine and ovarian sizes (Ibáñez et al. 2000, 2002a,b). These results have not been confirmed by other groups, however (Hernandez et al. 2006, Sadrzadeh et al. 2003, Jaquet et al. 1999, Legro et al. 2010).

A suboptimal intrauterine environment may trigger adverse effects on boys' gonadal development and increase the risk of cryptorchidism and hypospadias in the newborn (Weidner et al. 1999). These conditions can in turn increase the risk for poor semen quality and subsequently decrease fertility in adult

life (Jensen et al. 2004, Skakkebaek et al. 2001). In our cohort, cryptorchidism was evident in 2 boys (7.1%) and no hypospadias were found. The association between SGA and reproductive hormones in adolescent males is not as clear; one study found decreased testosterone and elevated LH levels in SGA men (Cicognani et al. 2002), whereas another study, in which cryptorchid boys were excluded, found no such association and concluded that SGA is not associated with adolescent male pituitary-testicular function (Jensen et al. 2007)

The impact of SGA on reproductive function also remains unknown. In 2010, Meas et al. investigated 579 adults born SGA from the general population and compared them to 703 subjects of the same age in terms of time to pregnancy (TTP). The results show that fertility was not reduced in subjects born SGA, and TTP was comparable in the two groups (Meas et al. 2010). In MUL, the basic genetic defect may affect the gonads (germ cells or supporting cells) directly, through general growth restraint, or in both of these ways.

6.6 Tumors in MUL

MUL is demonstrated here to be associated with an increased risk of benign and malignant tumors. In addition, signs of disturbed regulation of organ development were evident. A total of 232 tumorous lesions were revealed in 70 of the 92 patients (76%), meaning that some patients were diagnosed with more than one tumor, while others were not affected by any tumors at the time of examination. The majority of tumors (85%) were benign, including cysts, vascular lesions (hepatic peliosis), adrenal adenoma, parathyroid adenoma, thyroid goiter, pancreatic cystadenoma, renal angiomyolipoma, pheochromocytoma, and ovarian fibrothecomas. Of the patients, 15% exhibited malignant tumors, such as Wilms' tumor, renal papillary carcinoma, thyroid papillary and medullar carcinoma, ovarian and endometrial carcinoma, as well as acute lymphoblastic leukemia.

Tumorous lesions of the liver, as detected by ultrasound or MRI, were the most frequent tumors encountered in the MUL patients, often already present in young children. The lesions consisted of large, blood-filled and cyst-like cavities that interrupted the normal parenchyma (hepatic peliosis) (Tsokos and Erbersdobler 2005). Liver peliosis are rare and benign lesions that are often associated with an increased pressure in the hepatic circulation due to cardiac failure and pericardial constriction, also commonly seen in MUL. In addition, hepatic peliosis has been associated with many other medical conditions, including infections, alcohol and steroid abuse, diabetes mellitus, immunodeficiency, and wasting illnesses (Tsokos and Erbersdobler 2005), of which diabetes and wasting are likewise seen in MUL (Karlberg et al. 2005, 2007).

Vascular anomalies were also apparent in other organs than the liver, such as the adrenal and thyroid glands, lymph nodes, lungs and brain, which contained cystic blood vessels. These lesions showed abundant positive staining for the endothelial marker CD34, which is expressed in both progenitor and mature endothelial cells (Pusztaszeri et al. 2006). Normally, CD34 staining is negative in hepatic sinusoids, but positive in hepatocellular carcinoma (Haratake and Scheuer 1990, Coston et al. 2008), reflecting an increased capillarization of the sinusoids (Dhillon et al. 1992). Benign lesions may occasionally show focal CD34 positivity, but the intense staining seen in MUL, both in the liver and the lymph nodes, is clearly aberrant, and suggestive of a significantly disturbed angiogenesis (de Boer et al. 2000, Pusztaszeri et al. 2006, Hes and Morreau 2009). The weak staining for the proliferation marker MIB-1 is in line with the benign nature of the vascular lesions.

Several organs also showed disturbed architecture and ectopic tissues, implying a defective control of cell migration. For example, some MUL kidneys contained ovarian or adrenal tissues. Ectopic or accessory adrenal tissues are generally found along the path of descent of the gonads, but extremely rarely in the kidneys (Lack et al. 1997). In addition, the structure of the adrenal gland was deformed in several MUL patients, displaying fetal-type architecture and mixing of cortical and medullary tissues. These findings, together with frequent organ anomalies, speak for a disturbed organogenesis in MUL, the molecular basis of which remains to be elucidated.

6.6.1 Ovarian Tumors in MUL

During the follow-up of female MUL patients, it was observed that in addition to POF many of the patients developed ovarian tumors. Of the females in our study, 55% presented with ovarian fibrothecomas. These tumors are in general fairly uncommon, comprising 1-4% of all ovarian tumors (Young and Scully 1984, Roth and Czernobilsky 2011). They originate from the ovarian stromal or thecal cells and are often hormonally active, which can induce endometrial hyperplasia and even endometrial adenocarcinoma by prolonged secretion of estrogen (Roth and Czernobilsky 2011). Fibrothecomas have, to our knowledge, not been described previously in any other condition causing POF, suggesting that the TRIM37 protein has an important role in the control of theca cell growth.

Fibromas are composed of spindle, round, or oval collagen-producing cells, which resemble fibroblasts. In thecomas, the tumor cells contain lipid, resemble those of theca interna, and may contain variable amounts of fibroblasts (Roth and Czernobilsky 2011). Thecomas may have fibroma-like areas and vice versa, and due to this overlap the tumors are often collectively referred to as fibrothecomas. These tumors usually occur in postmenopausal women (mean age 63 years) (Aboud 1997), but the tumors were early onset in MUL females (mean age 29 years).

In three of the patients who were later diagnosed with fibrothecoma, inhibin B concentrations were considerably elevated. Despite a clear clinical POF diagnosis, their FSH levels were normal at the time of the diagnosis, indicating inhibin B-mediated suppression of gonadotropins. Inhibin B is a useful serum marker for the diagnosis and follow-up of granulosa cell tumors (Geerts et al. 2009). Our results suggest that the inhibin B levels are also elevated at least in a proportion of patients with ovarian fibrothecomas.

More than half of the MUL-associated fibrothecomas were multifocal or bilateral, and in three cases the tumors recurred after primary ovary sparing surgery. In most cases the tumor was clearly defined from the non-neoplastic ovarian tissue. These features strongly suggest that the tumors represent true neoplasias and not merely hyperplasia of stromal cells or diffuse ovarian fibromatosis, which is a rare non-neoplastic disorder of unknown origin (Young and Scully 1984). Most MUL-associated fibrothecomas also expressed inhibin-alpha, a typical feature of sporadic fibrothecomas.

In addition to fibrothecomas, 18% (4/22) of the MUL females had epithelial gynecological tumors. Two had serous adenofibromas, which are benign tumors composed of a fibroma-like stromal component and glandular structures of ciliated epithelium resembling the Fallopian tubes. Their molecular background and association with malignant epithelial ovarian neoplasias are unknown. One patient had a poorly differentiated ovarian adenocarcinoma diagnosed at the age of 33, suggesting that females with MUL could be predisposed to ovarian carcinoma, as well. Ovariectomy due to fibrothecomas and a shortened life expectancy due to the MUL heart disease (Lipsanen-Nyman et al. 2003) may prevent this feature from becoming more evident. One patient presented with a uterine endometrial adenocarcinoma and a concomitant new thecoma 9 years after her other ovary had been removed due to a thecoma. This case could presumably be explained by thecoma-associated excessive secretion of estrogen, a well-established risk factor for endometrial adenocarcinoma.

In addition to MUL, ovarian sex cord-stromal tumors are also found in several other inherited disorders. Gorlin syndrome (basal cell nevus syndrome; OMIM 109400), is an autosomal dominant disorder caused by mutations in the human homologue of *Drosophila* patched gene (*PTCH*) at 9q22.3 (Hahn et al. 1996). This disorder is characterized by multiple basal cell carcinomas of the skin, medulloblastoma, and ovarian fibromas, which are often bilateral and occur at a mean age of 30 years. Sotos syndrome (cerebral gigantism; OMIM 117550), caused by mutations in the *NSD1* gene (nuclear receptor binding SET domain protein 1) (Kurotaki et al. 2002), is another pleiotropic congenital syndrome where ovarian fibromas have been described (Chen et al. 2002). Patients with Peutz-Jeghers syndrome (PJS; OMIM 175200), caused by mutations of the *STK11* gene (serine/threonine kinase 11) at 19p13.3 (Hemminki et al. 1998), are susceptible to sex cord-stromal tumors with annular tubules (Young et al. 1982).

The molecular pathogenesis of sporadic fibrothecomas is unknown. Trisomy or tetrasomy of chromosome 12 is the most common, and often the only, genetic aberration detected by cytogenetic studies in these tumors (Fletcher et al. 1991, Liang et al. 2001). A less common finding is monosomy of chromosome 22 (Dal Cin et al. 1997), and also imbalances in chromosomes 4, 9, 10, and 18 have been found in this group of tumors (Micci et al. 2008, Streblow et al. 2007). Allelic imbalance (LOH) at 9q22.3 and 19p13.3 suggests the possible involvement of *PTCH* and *STK11* genes in the pathogenesis of sporadic fibrothecomas (Tsuji et al. 2005, Connolly et al. 2000, Kato et al. 2004), however no mutations of *STK11* have been identified in this group of tumors to date (Connolly et al. 2000, Kato et al. 2004). To our knowledge, LOH involving chromosome 17, site of *TRIM37*, has not been previously reported in the thecoma-fibroma group.

6.7 MUL- a Familial Cancer Syndrome?

Classic cancer syndromes, like Von Hippel-Lindau (VHL), typically show a clear recognizable spectrum of tumors and generally the tumors also occur at an early age. In MUL, the malignant tumors do not chiefly fit into this picture (Hes and Morreau 2009). For example, Wilms' tumors in MUL were diagnosed at a median age of 2.5 years, which is the same age as sporadic Wilms' tumors are detected (Scott et al. 2006, 2008). Nonetheless, the incidence of Wilms' tumor in MUL is clearly elevated (6.5%). In addition, tumors in classic cancer syndromes are typically bilateral and multifocal, which also do not apply to the malignant MUL tumors. The benign tumors in MUL, however, show several features typical of classic cancer syndromes, for example the ovarian fibrothecomas appeared at an unusually early age (29 years versus the mean age of 63 described in the literature), and the fibrothecomas were also often bilateral and multifocal. Renal cysts were also often seen in both kidneys and two patients had bilateral adrenocortical adenomas. In addition, some patients presented with more than one tumor, another feature typical for cancer syndromes.

There are several examples of genes responsible for hereditary cancer syndromes that are defective in sporadic tumors with similar morphology and location. The germ line harbors one allele with loss-of-function mutation and the other allele is inactivated by mutation, allele loss, or epigenetic events like promoter hypermethylation or translational down-regulation. The high frequency of fibrothecomas in the female MUL patients prompted investigation here of the *TRIM37* gene and protein status in sporadic fibrothecomas.

No *TRIM37* mutations were found in the sporadic fibrothecomas by sequencing the whole coding region; however, most sporadic fibrothecomas showed reduced or absent *TRIM37* protein expression in immunohistochemical analyses. The *TRIM37* transcript was detected in all sporadic fibrothecomas examined, but 80% (20/25) of the tumors showed reduced or absent expression of the *TRIM37* protein indicating alterations at the translational level. Furthermore,

LOH analysis and methylation studies also pointed towards a pathogenic association between TRIM37 alterations and development of sporadic fibrothecomas.

Interestingly, one male MUL patient developed a testicular thecoma at the age of 24 years. Thecomas are extremely rare in the testis, this being, to our knowledge, only the third case reported in the literature (Schenkman et al. 1983, Ueda et al. 2010). The second described thecoma in a male was in association with Gorlin syndrome, in which women also are predisposed to thecomas and fibromas. The occurrence of a thecoma in the MUL males suggests a possible pathogenic association between alterations in TRIM37 and thecoma-formation also in men.

Our results show that mutations of *TRIM37* predispose to both mesenchymal and epithelial ovarian tumors and suggest a possible role for TRIM37 in the development of sporadic tumors of the thecoma-fibroma group, implying a role for TRIM37 in tumorigenesis. Nonetheless, whether MUL will obtain a place among the established cancer syndromes in the future, remains to be seen.

6.8 Pathogenic Mechanisms of Tumor Development in MUL

The molecular basis of the tumor development in MUL remains elusive. TRIM proteins regulate many important cellular functions, such as insulin signaling, cell proliferation, differentiation, angiogenesis, apoptosis, and oncogenesis (Meroni and Diez-Roux 2005, Sun 2006, Rome et al. 2004, Hatakeyama 2011). Several TRIM proteins are involved in carcinogenesis and cancer progression (Hatakeyama 2011), and TRIM37 can be added to this list. Recently, it has been recognized that the majority of TRIM proteins function as E3 ubiquitin ligases (Borden 2000, Hatakeyama 2011), including TRIM37 (Kallijärvi et al. 2005). In general, cancer may develop from either stabilization of oncoproteins or destabilization of tumor suppressor genes. Interestingly, a growing number of cancer-associated proteins have been connected to the ubiquitin-proteasome pathway. Defective proteasomal degradation can either enhance the effect of oncoproteins or reduce the amount of tumor suppressor proteins, and to date, many oncogenes and tumor suppressor proteins have been found to be targets of ubiquitination (Mani and Gelmann 2005).

Von Hippel-Lindau syndrome (VHL) is a hereditary cancer syndrome caused by mutations in the *VHL* tumor suppressor gene (Latif et al. 1993), encoding for the E3 ligase pVHL, which ubiquitinates HIF-1 α during normoxia (Maxwell et al. 1999, Paul 2008). Loss of pVHL function leads to constitutive activation of HIF-1 α , resulting in an increased expression of hypoxia-inducible genes, including *VEGF* (vascular endothelial growth factor), promoting tumor growth and angiogenesis (Jung et al. 2006). Accordingly, the tumors associated with VHL have a highly vascular phenotype. Most tissues and cell types express pVHL,

suggesting that loss of pVHL may also generally be involved in tumor formation and angiogenesis (Jung et al. 2006).

MUL and VHL share some interesting histological and radiological features and in both conditions patients are predisposed to cystic and vascular lesions, especially in endocrine tissues. The tumor spectra in VHL and MUL, however, also have clear differences; VHL patients commonly develop hemangioblastomas, highly vascular benign tumors composed of stromal cells and blood vessels, primarily in the retina, cerebellum, and spinal cord (Kaelin 2007), while peliotic lesions in the liver are the most frequent tumor type in MUL. In addition, renal cell carcinoma is the most common malignant tumor in VHL (Kaelin 2007), in contrast to papillary carcinoma or Wilms' tumor in MUL. However, like in VHL, loss of TRIM37 seems to result in an increased angiogenesis and tumor formation. Whether this is due to an overexpression of VEGF or stimulation of some other angiogenic pathway, remains to be elucidated.

TRIM proteins can thus regulate oncogenesis and tumor progression positively or negatively by influencing pathways such as cell proliferation, DNA repair, and apoptosis. Recent data also indicate that TRIM proteins are involved in epigenetic regulation, suggesting that they could also contribute to tumor suppression or development by indirectly regulating gene expression (Hatakeyama 2011). Further understanding of the UPS and E3 ligases, including TRIM proteins, may thus generate effective targets for novel cancer therapies in the future. The exact role of TRIM37 in tumorigenesis will hopefully be clarified in future studies.

In conclusion, the pathogenesis underlying MUL remains elusive. The finding that TRIM37 acts as a ubiquitin E3 ligase (Kallijärvi et al. 2005) is important and offers an explanation for the pleiotropic features of the disease. The ubiquitin-proteasome system (UPS) is significant for normal cell function as it regulates normal protein turnover (Hershko and Ciechanover 1998). Apart from tumor formation (Sun 2006), impaired ubiquitination has also been implicated for example in prenatal growth failure (Sarikas et al. 2008), type 2 diabetes (Casas et al. 2007), and cardiomyopathy (Zolk et al. 2006), all common features in MUL. Recent evidence also suggests an important role for ubiquitination in gametogenesis and fertilization. The ubiquitin enzymes, including the E3 ligases, are important during spermatogenesis (Sutovsky 2003), and in the mammalian oocyte, the UPS has been implicated in oocyte meiosis (Verlhac et al. 2010), indicating a role for the UPS in gametogenesis and hence fertility. Future studies will hopefully shed light on the exact role for TRIM37 in the UPS and its implications in development and disease.

6.9 Limitations of the Study

Finnish MUL patients genotypically constitute a very homogenous group. Although no genotype-phenotype correlation has so far been demonstrated in MUL, it is possible that different *TRIM37* mutations do not lead to the same hypogonadal and tumor predisposing phenotype. As all published *TRIM37* mutations to date seem to produce a non-functioning protein, however, it is likely that the phenotypes seen in our patients are not only restricted to the Finnish genotypes.

Despite the fact that our MUL cohort is the largest in the world, the number of patients is still small and the age variation was large (0.7-77 years). Owing to the small number of patients, statistical analyses could not be performed. In studies I and II, when assessing the gonadal function in MUL, age- and sex matched controls would have been valuable. In addition, a larger sample size of testicular controls would have been valuable in study II, but testicular biopsies from healthy controls are very scarce as testicular biopsies are only taken on clinical grounds.

6.10 Future Prospects

Further elucidation of the development and timing of the gonadal failure in MUL will hopefully shed light on molecular pathways important for sexual maturation and fertility. As assisted reproductive technologies continue to develop, biological paternity will probably be possible for more MUL men, but the question of biological maternity in MUL still must be considered with much caution. The possibility of adoption should also be kept in mind for this patient group.

In patients with MUL, genetic defects in the *TRIM37* protein are associated with growth restriction, cardiopathy, early onset metabolic syndrome, hypogonadism, infertility, defective organogenesis and tumor formation. In the future, expanding the clinical studies to mouse models, and generation and characterization of induced pluripotent stem cells (iPS) from MUL patients, will offer a unique opportunity to study central cellular functions associated with the diverse organ manifestations of MUL as well as develop therapies for these patients.

7. CONCLUSIONS

In this study, a well-characterized study population of Mulibrey nanism (MUL) patients with mutations in the *TRIM37* gene were assessed for sexual maturation, gonadal function, and tumor predisposition.

The main conclusions of this study are:

1. Female and male hypogonadism, associated with a substantial risk for infertility, is a central clinical characteristic of MUL. Gonadal failure should be added to the list of major diagnostic signs in MUL.
2. Female patients with MUL demonstrate incomplete breast development, hypoplastic ovaries, a small uterus, and early irregularity of menstrual periods. All MUL females eventually develop premature ovarian failure (POF) and no female subject has become pregnant.
3. Male patients with MUL develop a unique disorder of testicular function characterized by small testes, elevated FSH and LH levels, and low inhibin B. No spontaneous pregnancies have been conceived among the MUL men; however, IVF by intracytoplasmic sperm injection (ICSI) gives hope for biological paternity in MUL, and to date, one MUL man has successfully undergone this treatment, resulting in the delivery of a healthy child.
4. *TRIM37* represents a new gene associated with POF and testicular failure and mutations in *TRIM37* can be added to the list of single-gene defects causing human infertility.
5. MUL patients show a high frequency of both benign and malignant tumors in several organs. They are especially prone to benign vascular lesions of the liver, hepatic peliosis, and adenomatous lesions. Organogenesis is also commonly disturbed.
6. Female MUL patients have a high risk of developing benign ovarian stromal tumors, fibrothecomas, and should thus undergo regular gynecological evaluations.

More precise knowledge of the function of the *TRIM37* gene will likely increase our understanding of the cellular functions regulating gametogenesis, gonadal function, proliferation, migration, and angiogenesis. MUL can thus be regarded as a model disease for infertility and tumor formation. Consequently, the study of rare monogenic disorders is not merely a treasure trove for elucidating gene function and for defining normal and pathological metabolic pathways, but it can also contribute significantly to our understanding of the molecular background of common, complex diseases.

8. ACKNOWLEDGEMENTS

This study was carried out at the Children's Hospital, University of Helsinki and Helsinki University Central Hospital. I wish to express my sincere appreciation to: Docent Jari Petäjä and Dr. Veli Ylitalo (the current and former Directors of the Department of Gynecology and Pediatrics, Helsinki University Central Hospital); Professor Mikael Knip (former Chair of the Children's Hospital, University of Helsinki); Docent Eero Jokinen (Head of the Department of Pediatrics, Helsinki University Central Hospital) and Professor Emeritus Christer Holmberg. They have provided excellent research facilities for the completion of this thesis study. Furthermore, Professor Markku Heikinheimo (Head of the Institute of Clinical Medicine and former Director of the Pediatric Graduate School) and Docent Jussi Merenmies (the present Director of the Pediatric Graduate School) are gratefully acknowledged for their provision of my scientific education and also for creating a supportive and positive research environment. In addition, Professor Markku Heikinheimo is warmly thanked for his encouragement, interest and support throughout the years.

Financial support for this work was gratefully received from Finska Läkaresällskapet, the Foundation for Pediatric Research, Medicinska Understödsföreningen Liv och Hälsa, the Finnish Medical Foundation, The Helsinki University Central Hospital Research Funds, the Päivikki and Sakari Sohlberg Foundation, the Academy of Finland and the Sigrid Juselius Foundation.

My supervisors, Docent Marita Lipsanen-Nyman and Professor Hannu Jalanko, I am tremendously grateful. Thank you Marita for entrusting me with the unique research material of the MUL disorder and for always believing in me and supporting my decisions. Many of the findings in this thesis are based on your clinical expertise and scientific observations of this fascinating disorder. Hannu, I wish to warmly thank you for guiding me through these last and most intense years of research, in which I learnt to believe in myself as a researcher. Despite your busy schedule, you amazingly always find the time to sit down and really listen to your students. Marita and Hannu, I feel privileged to have been under your wise, patient, and optimistic guidance, and I thank you for everything you have taught me - in science, medicine and life in general.

I am grateful to Docent Kirsti Näntö-Salonen and Professor Hannu Sariola for their skillful and timely review of the thesis, for valuable advice, and for inspiring discussions. In addition, I warmly thank Professor Hannu Sariola and Docent Outi Mäkitie for support and encouragement as members of my thesis committee. I also wish to specifically thank Outi for her positive and warm attitude, for all the support and help she has given me during the past years, and for always emphasizing the importance of life outside of work.

I warmly thank all my collaborators. Docent Ralf Bützow for his valuable contribution and for teaching me the basics of scientific reasoning and writing. Professor Aila Tiitinen for encouragement, support and friendly advice during the years, both in research and life outside of it. Aila is also warmly thanked for reading through selected parts of the thesis draft. Docent Heini Lassus for advice, friendship, laughter, and encouragement during the years. Dr. Riitta Karikoski for many hours spent by the microscope, for friendship and encouragement. Professor Jorma Toppari for inspiring discussions and helpful advice and for always finding the time to help no matter how small or big the problem ahead. M.Sc. Mirja Nurmio for helping with the analysis of the testicular samples, and for enjoyable times in both Turku and in Stockholm. Professor Anna-Elina Lehesjoki and the whole Folkhälsan MUL group; M.Sc. Jukka Kallijärvi, M.Sc. Riikka Hämäläinen, Dr. Kristiina Avela, and M.Sc. Kaisa Kettunen for collaboration, friendly advice and support. Dr. Sakari Mikkola for help in the field of radiology. Dr. Niklas Karlberg for collaboration in the tumor study and for help and advice throughout the years.

I warmly thank the research colleagues at the Research Laboratory of the Department of Obstetrics and Gynecology for advice and interest during study IV, in particular M.Sc. Sippy Kaur for friendship and support and Gynel Arifdshan for patiently teaching me the basics of laboratory work and for friendship.

I am grateful to Professor Emeritus Jaakko Perheentupa for his pivotal role in characterizing Mulibrey nanism and for interest and support during the years.

I warmly thank the doctors, nurses, and other personnel at the Division of Pediatric Endocrinology for interest in my research and for help and support. Anna-Liisa Silvennoinen for her practical help with the Mulibrey project, for kindness and friendship. Hanna-Kaisa Laine, Lea Schmidtman, Anu Niemi and Nea Boman for skillful and pleasant co-operation.

I warmly thank all the research colleagues at Biomedicum 2C sixth floor for inspiring discussions in addition to relaxing lunch and coffee breaks. I especially want to thank Mervi Mäyränpää for peer support and advice during the last and most intensive months of finalizing the thesis. Tuike Helmiö is warmly thanked for teaching me the basics of immunohistochemical staining, for expert help in the lab, and for kind friendship. The personnel at the Research Laboratory of the Children's Hospital are thanked for friendly advice and interest.

I am grateful to Helena Schmidt for expert and rapid help with my figures in this thesis and throughout the years.

I thank the clinical photographers of the Helsinki University Central Hospital for skillful contribution.

I warmly thank Dr. Jennifer Rowland for efficient language revision of the thesis.

I humbly and warmly thank all the MUL patients and their families for making this study possible and for their positive attitude towards research. Your joy of life and courage, despite living with a serious disorder like MUL, sets an example for all of us. I also want to thank the patients' local clinicians, surgeons, and pathologists for excellent collaboration.

I thank my lovely friends and their families outside of work, no one mentioned and no one forgotten, for heart-warming times, encouragement, and friendship throughout the years.

I warmly thank my parents-in-law, Solveig and Nils-Erik, for including me in their family and for caring and help with baby-sitting during the years. My sister-in-law Annika and her family are thanked for friendship and many joyful moments together. My uncle Juhani, his wife Katariina and their family are warmly thanked for support and caring.

I am deeply grateful to my family for continuous love and support. My mother Marita, for a safe and loving childhood, and for tremendous support and help in taking care of the children whenever needed. My father Jan-Anders, for awakening my early interest in books and learning and for proofreading the thesis manuscript. My sweet "little" brother Anders, the best brother anyone could ask for. I thank you for your love, support, and friendship.

I feel blessed to have such a wonderful family of my own. This work is dedicated to you. My beloved husband Niklas, my soul mate and best friend. I thank you for your love, support, and belief in me, for making me laugh, and for putting things, like this thesis, in to the right perspective. I love you. My precious, beautiful children, Nicole, Axel, and the little one still growing inside me. You are the single most important thing in my life. Mamma älskar er.

Helsinki, May 2012

Susann Karlberg

9. REFERENCES

- Aboud E. A review of granulosa cell tumours and thecomas of the ovary. *Arch Gynecol Obstet* 1997;259:161-165.
- Abu-Amero S, Monk D, Frost J, Preece M, Stanier P, Moore GE. The genetic aetiology of Silver-Russell syndrome. *J Med Genet* 2008;45:193-199.
- Achermann JC, Ito M, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. *Nat Genet* 1999;22:125-126.
- Achermann JC, Jameson JL. Fertility and infertility: genetic contributions from the hypothalamic-pituitary-gonadal axis. *Mol Endocrinol* 1999;13:812-818.
- Aittomäki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, Kaskikari R, Sankila EM, Lehväslaiho H, Engel AR, Nieschlag E, Huhtaniemi I, de la Chapelle A. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell* 1995;82:959-968.
- Allanson JE. Noonan syndrome. *Am J Med Genet C Semin Med Genet* 2007;145C:274-279.
- Allen EG, Sullivan AK, Marcus M, Small C, Dominguez C, Epstein MP, Charen K, He W, Taylor KC, Sherman SL. Examination of reproductive aging milestones among women who carry the FMR1 premutation. *Hum Reprod* 2007;22:2142-2152.
- Anasti JN. Premature ovarian failure: an update. *Fertil Steril* 1998;70:1-15.
- Andersson AM, Juul A, Petersen JH, Müller J, Groome NP, Skakkebaek NE. Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *J Clin Endocrinol Metab* 1997;82:3976-3981.
- Ankarberg-Lindgren C, Westphal O, Dahlgren J. Testicular size development and reproductive hormones in boys and adult males with Noonan syndrome: a longitudinal study. *Eur J Endocrinol* 2011;165:137-144.
- Atwood CS, Meethal SV, Liu T, Wilson AC, Gallego M, Smith MA, Bowen RL. Dysregulation of the hypothalamic-pituitary-gonadal axis with menopause and andropause promotes neurodegenerative senescence. *J Neuropathol Exp Neurol* 2005;64:93-103.
- Auerbach AD, Verlander PC. Disorders of DNA replication and repair. *Curr Opin Pediatr* 1997;9:600-616.
- Avela K, Lipsanen-Nyman M, Idänheimo N, Seemanova E, Rosengren S, Mäkelä TP, Perheentupa J, Chapelle AD, Lehesjoki AE. Gene encoding a new RING-B-box-Coiled-coil protein is mutated in mulibrey nanism. *Nat Genet* 2000;25:298-301.
- Avela K, Lipsanen-Nyman M, Perheentupa J, Wallgren-Pettersson C, Marchand S, Faure S, Sistonen P, de la Chapelle A, Lehesjoki AE. Assignment of the mulibrey nanism gene to 17q by linkage and linkage-disequilibrium analysis. *Am J Hum Genet* 1997;60:896-902.
- Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LL, Kahrizi K, Najmabadi H, Smith RJ. Human male infertility caused by mutations in the CATSPER1 channel protein. *Am J Hum Genet* 2009;84:505-510.
- Baker TG. A Quantitative and Cytological Study of Germ Cells in Human Ovaries. *Proc R Soc Lond B Biol Sci* 1963;158:417-433.

- Barker DJ. Fetal origins of coronary heart disease. *BMJ* 1995;311:171-174.
- Barker DJ. Fetal growth and adult disease. *Br J Obstet Gynaecol* 1992;99:275-276.
- Barlow C, Hirotsume S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, Crawley JN, Ried T, Tagle D, Wynshaw-Boris A. Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell* 1996;86:159-171.
- Bashamboo A, Ferraz-de-Souza B, Lourenço D, Lin L, Sebire NJ, Montjean D, Bignon-Topalovic J, Mandelbaum J, Siffroi JP, Christin-Maitre S, Radhakrishna U, Rouba H, Ravel C, Seeler J, Achermann JC, McElreavey K. Human male infertility associated with mutations in NR5A1 encoding steroidogenic factor 1. *Am J Hum Genet* 2010;87:505-512.
- Belchetz PE, Barth JH, Kaufman JM. Biochemical endocrinology of the hypogonadal male. *Ann Clin Biochem* 2010;47:503-515.
- Bellin M, Sarafoglou K, Nathan B. Precocious Puberty. In: Sarafoglou K (Ed.), *Pediatric Endocrinology and Inborn Errors of Metabolism*, McGraw-Hill Companies, New York, USA. 2009.
- Bhasin S. Approach to the infertile man. *J Clin Endocrinol Metab* 2007;92:1995-2004.
- Bhasin S, Basaria S. Diagnosis and treatment of hypogonadism in men. *Best Pract Res Clin Endocrinol Metab* 2011;25:251-270.
- Bidet M, Bachelot A, Touraine P. Premature ovarian failure: predictability of intermittent ovarian function and response to ovulation induction agents. *Curr Opin Obstet Gynecol* 2008;20:416-420.
- Biro FM, Lucky AW, Huster GA, Morrison JA. Pubertal staging in boys. *J Pediatr* 1995;127:100-102.
- Bloom D. The syndrome of congenital telangiectatic erythema and stunted growth. *J Pediatr* 1966;68:103-113.
- Bloom D. Congenital telangiectatic erythema resembling lupus erythematosus in dwarfs; probably a syndrome entity. *AMA Am J Dis Child* 1954;88:754-758.
- Bodega B, Porta C, Crosignani PG, Ginelli E, Marozzi A. Mutations in the coding region of the FOXL2 gene are not a major cause of idiopathic premature ovarian failure. *Mol Hum Reprod* 2004;10:555-557.
- Boder E. Ataxia-telangiectasia: an overview. *Kroc Found Ser* 1985;19:1-63.
- Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab* 2003;88:622-626.
- Borden KL. RING domains: master builders of molecular scaffolds? *J Mol Biol* 2000;295:1103-1112.
- Bordini B, Rosenfield RL. Normal pubertal development: part II: clinical aspects of puberty. *Pediatr Rev* 2011;32:281-292.
- Borgström B, Hreinsson J, Rasmussen C, Sheikhi M, Fried G, Keros V, Fridström M, Hovatta O. Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab* 2009;94:74-80.
- Bosch AM. Classical galactosaemia revisited. *J Inherit Metab Dis* 2006;29:516-525.

- Bouilly J, Bachelot A, Broutin I, Touraine P, Binart N. Novel NOBOX loss-of-function mutations account for 6.2% of cases in a large primary ovarian insufficiency cohort. *Hum Mutat* 2011;32:1108-1113.
- Bradley JR, Pober JS. Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene* 2001;20:6482-6491.
- Bremner WJ, Millar MR, Sharpe RM, Saunders PT. Immunohistochemical localization of androgen receptors in the rat testis: evidence for stage-dependent expression and regulation by androgens. *Endocrinology* 1994;135:1227-1234.
- Broekmans FJ, Visser JA, Laven JS, Broer SL, Themmen AP, Fauser BC. Anti-Müllerian hormone and ovarian dysfunction. *Trends Endocrinol Metab* 2008;19:340-347.
- Broer SL, Eijkemans MJ, Scheffer GJ, van Rooij IA, de Vet A, Themmen AP, Laven JS, de Jong FH, Te Velde ER, Fauser BC, Broekmans FJ. Anti-mullerian hormone predicts menopause: a long-term follow-up study in normoovulatory women. *J Clin Endocrinol Metab* 2011;96:2532-2539.
- Bruce S, Hannula-Jouppi K, Peltonen J, Kere J, Lipsanen-Nyman M. Clinically distinct epigenetic subgroups in Silver-Russell syndrome: the degree of H19 hypomethylation associates with phenotype severity and genital and skeletal anomalies. *J Clin Endocrinol Metab* 2009;94:579-587.
- Buiting K. Prader-Willi syndrome and Angelman syndrome. *Am J Med Genet C Semin Med Genet* 2010;154C:365-376.
- Burger AM, Seth AK. The ubiquitin-mediated protein degradation pathway in cancer: therapeutic implications. *Eur J Cancer* 2004;40:2217-2229.
- Casas S, Gomis R, Gribble FM, Altirriba J, Knuutila S, Novials A. Impairment of the ubiquitin-proteasome pathway is a downstream endoplasmic reticulum stress response induced by extracellular human islet amyloid polypeptide and contributes to pancreatic beta-cell apoptosis. *Diabetes* 2007;56:2284-2294.
- Cassidy SB, Driscoll DJ. Prader-Willi syndrome. *Eur J Hum Genet* 2009;17:3-13.
- Castrillon DH, Miao L, Kollipara R, Horner JW, DePinho RA. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* 2003;301:215-218.
- Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A, Ninfa EG, Frey AZ, Gash DJ, Chow EP. Isolation of the bovine and human genes for Müllerian inhibiting substance and expression of the human gene in animal cells. *Cell* 1986;45:685-698.
- Chambon M, Orsetti B, Berthe ML, Bascoul-Mollevis C, Rodriguez C, Duong V, Gleizes M, Thenot S, Bibeau F, Theillet C, Cavaillès V. Prognostic significance of TRIM24/TIF-1α gene expression in breast cancer. *Am J Pathol* 2011;178:1461-1469.
- Chand AL, Harrison CA, Shelling AN. Inhibin and premature ovarian failure. *Hum Reprod Update* 2010;16:39-50.
- Chand AL, Ponnampalam AP, Harris SE, Winship IM, Shelling AN. Mutational analysis of BMP15 and GDF9 as candidate genes for premature ovarian failure. *Fertil Steril* 2006;86:1009-1012.
- Chandley AC. Genetic contribution to male infertility. *Hum Reprod* 1998;13 Suppl 3:76-83; discussion 84-88.
- Chen CP, Yang YC, Lin SP, Wang W, Chang CL, Chang KM. Bilateral calcified ovarian fibromas in a patient with Sotos syndrome. *Fertil Steril* 2002;77:1285-1287.

- Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, Cacciari E. Low birth weight for gestational age and subsequent male gonadal function. *J Pediatr* 2002;141:376-379.
- Clayton PE, Gill MS. Normal growth and its endocrine control. In: Brook CGD and Hindmarsh PC (Eds.), *Clinical Paediatric Endocrinology*, Fourth edition. Blackwell Science Ltd. 2001.
- Clifford SC, Maher ER. Von Hippel-Lindau disease: clinical and molecular perspectives. *Adv Cancer Res* 2001;82:85-105.
- Cocquet J, Pailhoux E, Jaubert F, Servel N, Xia X, Pannetier M, De Baere E, Messiaen L, Cotinot C, Fellous M, Veitia RA. Evolution and expression of FOXL2. *J Med Genet* 2002;39:916-921.
- Connolly DC, Katabuchi H, Cliby WA, Cho KR. Somatic mutations in the STK11/LKB1 gene are uncommon in rare gynecological tumor types associated with Peutz-Jegher's syndrome. *Am J Pathol* 2000;156:339-345.
- Conway GS, Payne NN, Webb J, Murray A, Jacobs PA. Fragile X premutation screening in women with premature ovarian failure. *Hum Reprod* 1998;13:1184-1187.
- Cordts EB, Christofolini DM, Dos Santos AA, Bianco B, Barbosa CP. Genetic aspects of premature ovarian failure: a literature review. *Arch Gynecol Obstet* 2011;283:635-643.
- Cortes D, Thorup J, Lindenberg S, Visfeldt J. Infertility despite surgery for cryptorchidism in childhood can be classified by patients with normal or elevated follicle-stimulating hormone and identified at orchidopexy. *BJU Int* 2003;91:670-674.
- Coston WM, Loera S, Lau SK, Ishizawa S, Jiang Z, Wu CL, Yen Y, Weiss LM, Chu PG. Distinction of hepatocellular carcinoma from benign hepatic mimickers using Glypican-3 and CD34 immunohistochemistry. *Am J Surg Pathol* 2008;32:433-444.
- Cotton JB, Rebelle C, Bosio A, Ladreyt-Ponchon JP, Maillet J. Familial intrauterine nanism with constrictive pericarditis, the MuLiBrEy syndrome. *Pediatric* 1988;43:197-203.
- Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986;67:604-606.
- Crinò A, Schiaffini R, Ciampalini P, Spera S, Beccaria L, Benzi F, Bosio L, Corrias A, Gargantini L, Salvatoni A, Tonini G, Trifiro G, Livieri C; Genetic Obesity Study Group of Italian Society of Pediatric endocrinology and diabetology (SIEDP). Hypogonadism and pubertal development in Prader-Willi syndrome. *Eur J Pediatr* 2003;162:327-333.
- Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, Bisceglia L, Zelante L, Nagaraja R, Porcu S, Ristaldi MS, Marzella R, Rocchi M, Nicolino M, Lienhardt-Roussie A, Nivelon A, Verloes A, Schlessinger D, Gasparini P, Bonneau D, Cao A, Pilia G. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. *Nat Genet* 2001;27:159-166.
- Cumming GR, Kerr D, Ferguson CC. Constrictive pericarditis with dwarfism in two siblings (mulibrey nanism). *J Pediatr* 1976;88:569-572.
- Cunningham GR, Toma SM. Clinical review: Why is androgen replacement in males controversial? *J Clin Endocrinol Metab* 2011;96:38-52.
- Dal Cin P, Qi H, Pauwels P, Backx C, Van den Berghe H. Monosomy 22 in a fibrothecoma. *Cancer Genet Cytogenet* 1997;99:129-131.

De Baere E, Dixon MJ, Small KW, Jabs EW, Leroy BP, Devriendt K, Gillerot Y, Mortier G, Meire F, Van Maldergem L, Courtens W, Hjalgrim H, Huang S, Liebaers I, Van Regemorter N, Touraine P, Praphanphoj V, Verloes A, Udar N, Yellore V, Chalukya M, Yelchits S, De Paepe A, Kuttann F, Fellous M, Veitia R, Messiaen L. Spectrum of FOXL2 gene mutations in blepharophimosis-ptosis-epicanthus inversus (BPES) families demonstrates a genotype--phenotype correlation. *Hum Mol Genet* 2001;10:1591-1600.

De Baere E, Lemerrier B, Christin-Maitre S, Durval D, Messiaen L, Fellous M, Veitia R. FOXL2 mutation screening in a large panel of POF patients and XX males. *J Med Genet* 2002;39:e43.

de Boer WB, Segal A, Frost FA, Sterrett GF. Can CD34 discriminate between benign and malignant hepatocytic lesions in fine-needle aspirates and thin core biopsies? *Cancer* 2000;90:273-278.

de Bruin JP, Dorland M, Bruinse HW, Spliet W, Nikkels PG, Te Velde ER. Fetal growth retardation as a cause of impaired ovarian development. *Early Hum Dev* 1998 ;51:39-46.

De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. *Lancet* 2010;376:911-921.

Deutinger J, Bartl W, Pfersmann C, Neumark J, Bernaschek G. Fetal kidney volume and urine production in cases of fetal growth retardation. *J Perinat Med* 1987;15:307-315.

Dhillon AP, Colombari R, Savage K, Scheuer PJ. An immunohistochemical study of the blood vessels within primary hepatocellular tumours. *Liver* 1992;12:311-318.

Dieterich K, Soto Rifo R, Faure AK, Hennebicq S, Ben Amar B, Zahi M, Perrin J, Martinez D, Sele B, Jouk PS, Ohlmann T, Rousseaux S, Lunardi J, Ray PF. Homozygous mutation of AURKC yields large-headed polyploid spermatozoa and causes male infertility. *Nat Genet* 2007;39:661-665.

Dieterich K, Zouari R, Harbuz R, Vialard F, Martinez D, Bellayou H, Prisant N, Zoghmar A, Guichaoua MR, Koscinski I, Kharouf M, Noruzinia M, Nadifi S, Sefiani A, Lornage J, Zahi M, Viville S, Sele B, Jouk PS, Jacob MC, Escalier D, Nikas Y, Hennebicq S, Lunardi J, Ray PF. The Aurora Kinase C c.144delC mutation causes meiosis I arrest in men and is frequent in the North African population. *Hum Mol Genet* 2009;18:1301-1309.

Dixit H, Deendayal M, Singh L. Mutational analysis of the mature peptide region of inhibin genes in Indian women with ovarian failure. *Hum Reprod* 2004;19:1760-1764.

Doğanc T, Yüksel Konuk BE, Alpan N, Konuk O, Hämäläinen RH, Lehesjoki AE, Tekin M. A novel mutation in TRIM37 is associated with mulibrey nanism in a Turkish boy. *Clin Dysmorphol* 2007;16:173-176.

Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 1996;383:531-535.

Dube JL, Wang P, Elvin J, Lyons KM, Celeste AJ, Matzuk MM. The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. *Mol Endocrinol* 1998;12:1809-1817.

Eiholzer U, l'Allemand D, Rousson V, Schlumpf M, Gasser T, Girard J, Gruters A, Simoni M. Hypothalamic and gonadal components of hypogonadism in boys with Prader-Labhart- Willi syndrome. *J Clin Endocrinol Metab* 2006;91:892-898.

Eldar-Geva T, Hirsch HJ, Benarroch F, Rubinstein O, Gross-Tsur V. Hypogonadism in females with Prader-Willi syndrome from infancy to adulthood: variable combinations of a primary gonadal defect and hypothalamic dysfunction. *Eur J Endocrinol* 2010;162:377-384.

Ellis NA, German J. Molecular genetics of Bloom's syndrome. *Hum Mol Genet* 1996;5 Spec No:1457-1463.

Ellis NA, Groden J, Ye TZ, Straughen J, Lennon DJ, Ciocci S, Proytcheva M, German J. The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 1995;83:655-666.

Eriksson JG, Forsén T, Tuomilehto J, Osmond C, Barker DJ. Early adiposity rebound in childhood and risk of Type 2 diabetes in adult life. *Diabetologia* 2003;46:190-194.

Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: role of genetic background. *Reprod Biomed Online* 2007;14:734-745.

Ferlin A, Vinanzi C, Garolla A, Selice R, Zuccarello D, Cazzadore C, Foresta C. Male infertility and androgen receptor gene mutations: clinical features and identification of seven novel mutations. *Clin Endocrinol (Oxf)* 2006;65:606-610.

Finni K, Herva R. Syndrome Mulibrey Nanism. A case report and autopsy findings in a 8-month-old girl (author's transl). *Klin Padiatr* 1981;193:328-330.

Finnish-German APECED Consortium. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat Genet* 1997;17:399-403.

Fletcher JA, Gibas Z, Donovan K, Perez-Atayde A, Genest D, Morton CC, Lage JM. Ovarian granulosa-stromal cell tumors are characterized by trisomy 12. *Am J Pathol* 1991;138:515-520.

Friedler S, Raziel A, Strassburger D, Schachter M, Bern O, Ron-El R. Outcome of ICSI using fresh and cryopreserved-thawed testicular spermatozoa in patients with non-mosaic Klinefelter's syndrome. *Hum Reprod* 2001;16:2616-2620.

Garber K, Smith KT, Reines D, Warren ST. Transcription, translation and fragile X syndrome. *Curr Opin Genet Dev* 2006;16:270-275.

Geerts I, Vergote I, Neven P, Billen J. The role of inhibins B and antimullerian hormone for diagnosis and follow-up of granulosa cell tumors. *Int J Gynecol Cancer* 2009;19:847-855.

Ghadami M, El-Demerdash E, Salama SA, Binhazim AA, Archibong AE, Chen X, Ballard BR, Sairam MR, Al-Hendy A. Toward gene therapy of premature ovarian failure: intraovarian injection of adenovirus expressing human FSH receptor restores folliculogenesis in FSHR(-/-) FORKO mice. *Mol Hum Reprod* 2010;16:241-250.

Gibson AT, Carney S, Wright NP, Wales JK. Measurement and the newborn infant. *Horm Res* 2003;59 Suppl 1:119-128.

Giles RH, Lolkema MP, Snijckers CM, Belderbos M, van der Groep P, Mans DA, van Beest M, van Noort M, Goldschmeding R, van Diest PJ, Clevers H, Voest EE. Interplay between VHL/HIF1alpha and Wnt/beta-catenin pathways during colorectal tumorigenesis. *Oncogene* 2006;25:3065-3070.

Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol* 2011;7:219-231.

Gosden RG, Faddy MJ. Biological bases of premature ovarian failure. *Reprod Fertil Dev* 1998;10:73-78.

Goswami D, Conway GS. Premature ovarian failure. *Hum Reprod Update* 2005;11:391-410.

Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, McNeilly AS. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1996;81:1401-1405.

Grootegoed JA, Siep M, Baarends WM. Molecular and cellular mechanisms in spermatogenesis. *Baillieres Best Pract Res Clin Endocrinol Metab* 2000;14:331-343.

Grumbach MM. The neuroendocrinology of human puberty revisited. *Horm Res* 2002;57 Suppl 2:2-14.

Grumbach MM. A window of opportunity: The diagnosis of gonadotropin deficiency in the male infant. *J Clin Endocrinol Metab.* 2005;90:3122-3127.

Gubbels CS, Land JA, Rubio-Gozalbo ME. Fertility and impact of pregnancies on the mother and child in classic galactosemia. *Obstet Gynecol Surv* 2008;63:334-343.

Gunay-Aygun M, Schwartz S, Heeger S, O'Riordan MA, Cassidy SB. The changing purpose of Prader-Willi syndrome clinical diagnostic criteria and proposed revised criteria. *Pediatrics* 2001;108:E92.

Gärtner J, Braun A, Holzinger A, Roerig P, Lenard HG, Roscher AA. Clinical and genetic aspects of X-linked adrenoleukodystrophy. *Neuropediatrics* 1998;29:3-13.

Hahn H, Wicking C, Zaphiropoulos PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toftgard R, Chenevix-Trench G, Wainwright B, Bale AE. Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome. *Cell* 1996;85:841-851.

Hainaut P, Hollstein M. P53 and Human Cancer: the First Ten Thousand Mutations. *Adv Cancer Res* 2000;77:81-137.

Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001;60:5-20.

Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992;35:595-601.

Hall JG. Review and hypothesis: syndromes with severe intrauterine growth restriction and very short stature--are they related to the epigenetic mechanism(s) of fetal survival involved in the developmental origins of adult health and disease? *Am J Med Genet A* 2010;152A:512-527.

Hanley NA, Ball SG, Clement-Jones M, Hagan DM, Strachan T, Lindsay S, Robson S, Ostrer H, Parker KL, Wilson DI. Expression of steroidogenic factor 1 and Wilms' tumour 1 during early human gonadal development and sex determination. *Mech Dev* 1999;87:175-180.

Hannula K, Kere J, Pirinen S, Holmberg C, Lipsanen-Nyman M. Do patients with maternal uniparental disomy for chromosome 7 have a distinct mild Silver-Russell phenotype? *J Med Genet* 2001;38:273-278.

Hansen PF, With TK. Clinical measurements of the testes in boys and men. *Acta Med Scand Suppl* 1952;266:457-465.

Haraldsson A, van der Burgt CJ, Weemaes CM, Otten B, Bakkeren JA, Stoelinga GB. Antibody deficiency and isolated growth hormone deficiency in a girl with Mulibrey nanism. *Eur J Pediatr* 1993;152:509-512.

Haratake J, Scheuer PJ. An immunohistochemical and ultrastructural study of the sinusoids of hepatocellular carcinoma. *Cancer* 1990;65:1985-1993.

Harbuz R, Zouari R, Pierre V, Ben Khelifa M, Kharouf M, Coutton C, Merdassi G, Abada F, Escoffier J, Nikas Y, Vialard F, Kosciński I, Triki C, Sermondade N, Schweitzer T, Zhioua A, Zhioua F, Latrous H, Halouani L, Ouafi M, Makni M, Jouk PS, Sele B, Hennebicq S, Satre V, Viville S, Arnoult C, Lunardi J, Ray PF. A recurrent deletion of DPY19L2 causes infertility in man by blocking sperm head elongation and acrosome formation. *Am J Hum Genet* 2011;88:351-361.

Harris SE, Chand AL, Winship IM, Gersak K, Aittomäki K, Shelling AN. Identification of novel mutations in FOXL2 associated with premature ovarian failure. *Mol Hum Reprod* 2002;8:729-733.

- Hatakeyama S. TRIM proteins and cancer. *Nat Rev Cancer* 2011;11:792-804.
- Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997;387:296-299.
- Hawkins SM, Matzuk MM. The menstrual cycle: basic biology. *Ann N Y Acad Sci* 2008;1135:10-18.
- Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, Bignell G, Warren W, Aminoff M, Höglund P, Järvinen H, Kristo P, Pelin K, Ridanpää M, Salovaara R, Toro T, Bodmer W, Olschwang S, Olsen AS, Stratton MR, de la Chapelle A, Aaltonen LA. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature* 1998;391:184-187.
- Hernandez MI, Martinez A, Capurro T, Pena V, Trejo L, Avila A, Salazar T, Asenjo S, Iniguez G, Mericq V. Comparison of clinical, ultrasonographic, and biochemical differences at the beginning of puberty in healthy girls born either small for gestational age or appropriate for gestational age: preliminary results. *J Clin Endocrinol Metab* 2006;91:3377-3381.
- Herquel B, Ouararhni K, Khetchoumian K, Ignat M, Teletin M, Mark M, Bechade G, Van Dorsselaer A, Sanglier-Cianferani S, Hamiche A, Cammas F, Davidson I, Losson R. Transcription cofactors TRIM24, TRIM28, and TRIM33 associate to form regulatory complexes that suppress murine hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 2011;108:8212-8217.
- Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 1998;67:425-479.
- Hershko A, Ciechanover A. The ubiquitin system for protein degradation. *Annu Rev Biochem* 1992;61:761-807.
- Hershko A, Ciechanover A. Mechanisms of intracellular protein breakdown. *Annu Rev Biochem* 1982;51:335-364.
- Hes FJ, Morreau H. Where genetics and pathology meet: mulibrey nanism. *J Pathol* 2009;218:143-145.
- Hicke L, Dunn R. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu Rev Cell Dev Biol* 2003;19:141-172.
- Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, Anderson KC. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res* 2001;61:3071-3076.
- Hiort O, Holterhus PM, Horter T, Schulze W, Kremke B, Bals-Pratsch M, Sinnecker GH, Kruse K. Significance of mutations in the androgen receptor gene in males with idiopathic infertility. *J Clin Endocrinol Metab* 2000;85:2810-2815.
- Hiort O, Sinnecker GH, Holterhus PM, Nitsche EM, Kruse K. The clinical and molecular spectrum of androgen insensitivity syndromes. *Am J Med Genet* 1996;63:218-222.
- Hitchins MP, Stanier P, Preece MA, Moore GE. Silver-Russell syndrome: a dissection of the genetic aetiology and candidate chromosomal regions. *J Med Genet* 2001;38:810-819.
- Hollstein M, Sidransky D, Vogelstein B, Harris CC. P53 Mutations in Human Cancers. *Science* 1991;253:49-53.
- Honda R, Tanaka H, Yasuda H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett* 1997;420:25-27.
- Hovatta O. Pregnancies in women with Turner's syndrome. *Ann Med* 1999;31:106-110.

Hreinsson JG, Otala M, Fridström M, Borgström B, Rasmussen C, Lundqvist M, Tuuri T, Simberg N, Mikkola M, Dunkel L, Hovatta O. Follicles are found in the ovaries of adolescent girls with Turner's syndrome. *J Clin Endocrinol Metab* 2002;87:3618-3623.

Hsueh AJ, Billig H, Tsafiriri A. Ovarian follicle atresia: a hormonally controlled apoptotic process. *Endocr Rev* 1994;15:707-724.

Hsueh AJ, Eisenhauer K, Chun SY, Hsu SY, Billig H. Gonadal cell apoptosis. *Recent Prog Horm Res* 1996;51:433-455; discussion 455-456.

Hämäläinen RH, Avela K, Lambert JA, Kallijärvi J, Eyaid W, Gronau J, Ignaszewski AP, McFadden D, Sorge G, Lipsanen-Nyman M, Lehesjoki AE. Novel mutations in the TRIM37 gene in Mulibrey Nanism. *Hum Mutat* 2004;23:522.

Hämäläinen RH, Mowat D, Gabbett MT, O'Brien TA, Kallijärvi J, Lehesjoki AE. Wilms' tumor and novel TRIM37 mutations in an Australian patient with mulibrey nanism. *Clin Genet* 2006;70:473-479.

Ibáñez L, Potau N, Enriquez G, de Zegher F. Reduced uterine and ovarian size in adolescent girls born small for gestational age. *Pediatr Res* 2000;47:575-577.

Ibáñez L, Potau N, Ferrer A, Rodriguez-Hierro F, Marcos MV, de Zegher F. Reduced ovulation rate in adolescent girls born small for gestational age. *J Clin Endocrinol Metab* 2002a;87:3391-3393.

Ibáñez L, Valls C, Miro E, Marcos MV, de Zegher F. Early menarche and subclinical ovarian hyperandrogenism in girls with reduced adult height after low birth weight. *J Pediatr Endocrinol Metab* 2002b;15:431-433.

Ikeda Y, Shen WH, Ingraham HA, Parker KL. Developmental expression of mouse steroidogenic factor-1, an essential regulator of the steroid hydroxylases. *Mol Endocrinol* 1994;8:654-662.

Illingworth PJ, Groome NP, Byrd W, Rainey WE, McNeilly AS, Mather JP, Bremner WJ. Inhibin-B: a likely candidate for the physiologically important form of inhibin in men. *J Clin Endocrinol Metab* 1996;81:1321-1325.

Isidori AM, Giannetta E, Lenzi A. Male hypogonadism. *Pituitary* 2008;11:171-180.

Jacquemont S, Hagerman RJ, Hagerman PJ, Leehey MA. Fragile-X syndrome and fragile X-associated tremor/ataxia syndrome: two faces of FMR1. *Lancet Neurol* 2007;6:45-55.

Jagiello P, Hammans C, Wiczorek S, Arning L, Stefanski A, Strehl H, Epplen JT, Gencik M. A novel splice site mutation in the TRIM37 gene causes mulibrey nanism in a Turkish family with phenotypic heterogeneity. *Hum Mutat* 2003;21:630-635.

Jaquet D, Leger J, Chevenne D, Czernichow P, Levy-Marchal C. Intrauterine growth retardation predisposes to insulin resistance but not to hyperandrogenism in young women. *J Clin Endocrinol Metab* 1999;84:3945-3949.

Jensen RB, Vielwerth S, Larsen T, Greisen G, Veldhuis J, Juul A. Pituitary-gonadal function in adolescent males born appropriate or small for gestational age with or without intrauterine growth restriction. *J Clin Endocrinol Metab* 2007;92:1353-1357.

Jensen TK, Jørgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B, Horte A, Andersen AG, Carlsen E, Magnus Ø, Matulevicius V, Nermoen I, Vierula M, Keiding N, Toppari J, Skakkebaek NE. Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1,770 young men from the general population in five European countries. *Am J Epidemiol* 2004;159:49-58.

Jeyasuria P, Ikeda Y, Jamin SP, Zhao L, De Rooij DG, Themmen AP, Behringer RR, Parker KL. Cell-specific knockout of steroidogenic factor 1 reveals its essential roles in gonadal function. *Mol Endocrinol* 2004;18:1610-1619.

Joazeiro CA, Weissman AM. RING finger proteins: mediators of ubiquitin ligase activity. *Cell* 2000;102:549-552.

Johnston DS, Russell LD, Friel PJ, Griswold MD. Murine germ cells do not require functional androgen receptors to complete spermatogenesis following spermatogonial stem cell transplantation. *Endocrinology* 2001;142:2405-2408.

Jørgensen N, Vierula M, Jacobsen R, Pukkala E, Perheentupa A, Virtanen HE, Skakkebaek NE, Toppari J. Recent adverse trends in semen quality and testis cancer incidence among Finnish men. *Int J Androl* 2011;34:e37-48.

Jung CR, Hwang KS, Yoo J, Cho WK, Kim JM, Kim WH, Im DS. E2-EPF UCP targets pVHL for degradation and associates with tumor growth and metastasis. *Nat Med* 2006;12:809-816.

Kaelin WG. Von Hippel-Lindau disease. *Annu Rev Pathol* 2007;2:145-173.

Kaelin WG, Jr. Molecular basis of the VHL hereditary cancer syndrome. *Nat Rev Cancer* 2002;2:673-682.

Kallijärvi J, Avela K, Lipsanen-Nyman M, Ulmanen I, Lehesjoki AE. The TRIM37 gene encodes a peroxisomal RING-B-box-coiled-coil protein: classification of mulibrey nanism as a new peroxisomal disorder. *Am J Hum Genet* 2002;70:1215-1228.

Kallijärvi J, Hämäläinen RH, Karlberg N, Sainio K, Lehesjoki AE. Tissue expression of the mulibrey nanism-associated Trim37 protein in embryonic and adult mouse tissues. *Histochem Cell Biol* 2006;126:325-334.

Kallijärvi J, Lahtinen U, Hämäläinen R, Lipsanen-Nyman M, Palvimo JJ, Lehesjoki AE. TRIM37 defective in mulibrey nanism is a novel RING finger ubiquitin E3 ligase. *Exp Cell Res* 2005;308:146-155.

Kallio S, Aittomäki K, Piltonen T, Veijola R, Liakka A, Vaskivuo TE, Dunkel L, Tapanainen JS. Anti-Müllerian hormone as a predictor of follicular reserve in ovarian insufficiency: special emphasis on FSH-resistant ovaries. *Hum Reprod* 2012;27:854-860.

Kaplan SL, Grumbach MM, Aubert ML. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus: maturation of central nervous system regulation of anterior pituitary function. *Recent Prog Horm Res* 1976;32:161-243.

Karlberg J. A biologically-oriented mathematical model (ICP) for human growth. *Acta Paediatr Scand Suppl* 1989;350:70-94.

Karlberg J, Engström I, Karlberg P, Fryer JG. Analysis of linear growth using a mathematical model. I. From birth to three years. *Acta Paediatr Scand* 1987a;76:478-488.

Karlberg J, Fryer JG, Engström I, Karlberg P. Analysis of linear growth using a mathematical model. II. From 3 to 21 years of age. *Acta Paediatr Scand Suppl* 1987b;337:12-29.

Karlberg N, Jalanko H, Kallijärvi J, Lehesjoki AE, Lipsanen-Nyman M. Insulin resistance syndrome in subjects with mutated RING finger protein TRIM37. *Diabetes* 2005;54:3577-3581.

Karlberg N, Jalanko H, Lipsanen-Nyman M. Growth and growth hormone therapy in subjects with mulibrey nanism. *Pediatrics* 2007;120:e102-111.

Karlberg N, Jalanko H, Perheentupa J, Lipsanen-Nyman M. Mulibrey nanism: clinical features and diagnostic criteria. *J Med Genet* 2004;41:92-98.

- Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 2004;432:316-323.
- Kato N, Romero M, Catusas L, Prat J. The STK11/LKB1 Peutz-Jegher gene is not involved in the pathogenesis of sporadic sex cord-stromal tumors, although loss of heterozygosity at 19p13.3 indicates other gene alteration in these tumors. *Hum Pathol* 2004;35:1101-1104.
- Kaufman FR, Donnell GN, Roe TF, Kogut MD. Gonadal function in patients with galactosae-mia. *J Inherit Metab Dis* 1986;9:140-146.
- Kaufman FR, Kogut MD, Donnell GN, Goebelsmann U, March C, Koch R. Hypergonadotropic hypogonadism in female patients with galactosemia. *N Engl J Med* 1981;304:994-998.
- Kelleher S, Conway AJ, Handelsman DJ. Blood testosterone threshold for androgen deficiency symptoms. *J Clin Endocrinol Metab* 2004;89:3813-3817.
- Kestilä M, Ikonen E, Lehesjoki AE. Finnish disease heritage. *Duodecim* 2010;126:2311-2320.
- Kevenaar ME, Meerasahib MF, Kramer P, van de Lang-Born BM, de Jong FH, Groome NP, Themmen AP, Visser JA. Serum anti-mullerian hormone levels reflect the size of the primordial follicle pool in mice. *Endocrinology* 2006;147:3228-3234.
- Kim WY, Kaelin WG. Role of VHL gene mutation in human cancer. *J Clin Oncol* 2004;22:4991-5004.
- Klinefelter HF, Reifenstein EC, Albright F. Syndrome characterized by gynecomastia, aspermatogenesis without a-Leydigism, and increased excretion of follicle-stimulating hormone. *Am J Clin Dermatol* 1942;2:615-627.
- Kojima Y, Sasaki S, Hayashi Y, Umemoto Y, Morohashi K, Kohri K. Role of transcription factors Ad4bp/SF-1 and DAX-1 in steroidogenesis and spermatogenesis in human testicular development and idiopathic azoospermia. *Int J Urol* 2006;13:785-793.
- Krausz C. Male infertility: pathogenesis and clinical diagnosis. *Best Pract Res Clin Endocrinol Metab* 2011;25:271-285.
- Krausz C, Degl'Innocenti S. Y chromosome and male infertility: update, 2006. *Front Biosci* 2006;11:3049-3061.
- Krsmanovic LZ, Hu L, Leung PK, Feng H, Catt KJ. The hypothalamic GnRH pulse generator: multiple regulatory mechanisms. *Trends Endocrinol Metab* 2009;20:402-408.
- Kuiri-Hänninen T, Seuri R, Tyrväinen E, Turpeinen U, Hämäläinen E, Stenman UH, Dunkel L, Sankilampi U. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. *J Clin Endocrinol Metab* 2011a;96:98-105.
- Kuiri-Hänninen T, Kallio S, Seuri R, Tyrväinen E, Liakka A, Tapanainen J, Sankilampi U, Dunkel L. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. *J Clin Endocrinol Metab*. 2011b;96:3432-3439.
- Kurjak A, Kirkinen P, Latin V, Ivankovic D. Ultrasonic assessment of fetal kidney function in normal and complicated pregnancies. *Am J Obstet Gynecol* 1981;141:266-270.
- Kurotaki N, Imaizumi K, Harada N, Masuno M, Kondoh T, Nagai T, Ohashi H, Naritomi K, Tsukahara M, Makita Y, Sugimoto T, Sonoda T, Hasegawa T, Chinen Y, Tomita Ha HA, Kinoshita A, Mizuguchi T, Yoshiura Ki K, Ohta T, Kishino T, Fukushima Y, Niikawa N, Matsumoto N. Haploinsufficiency of NSD1 causes Sotos syndrome. *Nat Genet* 2002;30:365-366.
- Lack EE, Bryan JA, Lewin-Smith M. Adrenal glands. In: Bostwick DG and Eble JN (Eds.), *Urologic Surgical Pathology*, Mosby, St. Louis, USA. 1997.

La Marca A, Pati M, Orvieto R, Stabile G, Carducci Artenisio A, Volpe A. Serum anti-müllerian hormone levels in women with secondary amenorrhea. *Fertil Steril* 2006;85:1547-1549.

Lapunzina P, Rodriguez JI, de Matteo E, Gracia R, Moreno F. Mulibrey nanism: three additional patients and a review of 39 patients. *Am J Med Genet* 1995;55:349-355.

Laron Z. Growth hormone insensitivity (Laron syndrome). *Rev Endocr Metab Disord* 2002;3:347-355.

Latif F, Tory K, Gnarr J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 1993;260:1317-1320.

Lavin MF. Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. *Nat Rev Mol Cell Biol* 2008;9:759-769.

Leder P, Battey J, Lenoir G, Moulding C, Murphy W, Potter H, Stewart T, Taub R. Translocations among antibody genes in human cancer. *Science* 1983;222:765-771.

Legro RS, Roller RL, Dodson WC, Stetter CM, Kunselman AR, Dunaif A. Associations of birth-weight and gestational age with reproductive and metabolic phenotypes in women with polycystic ovarian syndrome and their first-degree relatives. *J Clin Endocrinol Metab* 2010;95:789-799.

Leslie ND, Immerman EB, Flach JE, Florez M, Fridovich-Keil JL, Elsas LJ. The human galactose-1-phosphate uridylyltransferase gene. *Genomics* 1992;14:474-480.

Levine AJ. P53, the Cellular Gatekeeper for Growth and Division. *Cell* 1997;88:323-331.

Levron J, Aviram-Goldring A, Madgar I, Raviv G, Barkai G, Dor J. Sperm chromosome analysis and outcome of IVF in patients with non-mosaic Klinefelter's syndrome. *Fertil Steril* 2000;74:925-929.

Lewis K, Lee PA. Endocrinology of male puberty. *Curr Opin Endocrinol Diabetes Obes*. 2009;16:5-9.

Liang SB, Sonobe H, Taguchi T, Takeuchi T, Furihata M, Yuri K, Ohtsuki Y. Tetrasomy 12 in ovarian tumors of thecoma-fibroma group: A fluorescence in situ hybridization analysis using paraffin sections. *Pathol Int* 2001;51:37-42.

Lin L, Achermann JC. Steroidogenic factor-1 (SF-1, Ad4BP, NR5A1) and disorders of testis development. *Sex Dev* 2008;2:200-209.

Lipsanen-Nyman M. Mulibrey nanismi. Academic dissertation, University of Helsinki, Helsinki, Finland. 1986.

Lipsanen-Nyman M, Perheentupa J, Rapola J, Sovijärvi A, Kupari M. Mulibrey heart disease: clinical manifestations, long-term course, and results of pericardiectomy in a series of 49 patients born before 1985. *Circulation* 2003;107:2810-2815.

Los M, Jansen GH, Kaelin WG, Lips CJ, Blijham GH, Voest EE. Expression pattern of the von Hippel-Lindau protein in human tissues. *Lab Invest* 1996;75:231-238.

Lourenço D, Brauner R, Lin L, De Perdigo A, Weryha G, Muresan M, Boudjenah R, Guerra-Junior G, Maciel-Guerra AT, Achermann JC, McElreavey K, Bashamboo A. Mutations in NR5A1 associated with ovarian insufficiency. *N Engl J Med* 2009;360:1200-1210.

Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* 1994;77:481-490.

MacNaughton J, Banah M, McCloud P, Hee J, Burger H. Age related changes in follicle stimulating hormone, luteinizing hormone, oestradiol and immunoreactive inhibin in women of reproductive age. *Clin Endocrinol (Oxf)* 1992;36:339-345.

Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, Hirsch B, Jacky P, McDowell GA, Popovich B, Watson M, Wolff DJ. Technical standards and guidelines for fragile X: the first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. *Genet Med* 2001;3:200-205.

Manasco PK, Umbach DM, Muly SM, Godwin DC, Negro-Vilar A, Culler MD, Underwood LE. Ontogeny of gonadotrophin and inhibin secretion in normal girls through puberty based on overnight serial sampling and a comparison with normal boys. *Hum Reprod* 1997;12:2108-2114.

Mani A, Gelmann EP. The ubiquitin-proteasome pathway and its role in cancer. *J Clin Oncol* 2005;23:4776-4789.

Marcus KA, Sweep CG, van der Burgt I, Noordam C. Impaired Sertoli cell function in males diagnosed with Noonan syndrome. *J Pediatr Endocrinol Metab* 2008;21:1079-1084.

Marozzi A, Porta C, Vegetti W, Crosignani PG, Tibiletti MG, Dalpra L, Ginelli E. Mutation analysis of the inhibin alpha gene in a cohort of Italian women affected by ovarian failure. *Hum Reprod* 2002;17:1741-1745.

Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970;45:13-23.

Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child* 1969;44:291-303.

Mathis D, Benoist C. A decade of AIRE. *Nat Rev Immunol* 2007;7:645-650.

Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. *Science* 2002;296:2178-2180.

Matzuk MM, Finegold MJ, Su JG, Hsueh AJ, Bradley A. Alpha-inhibin is a tumour-suppressor gene with gonadal specificity in mice. *Nature* 1992;360:313-319.

Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 1999;399:271-275.

McLachlan RI, O'Bryan MK. Clinical Review#: State of the art for genetic testing of infertile men. *J Clin Endocrinol Metab* 2010;95:1013-1024.

McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de K, Pratis K, Robertson DM. Hormonal regulation of spermatogenesis in primates and man: insights for development of the male hormonal contraceptive. *J Androl* 2002a;23:149-162.

McLachlan RI, O'Donnell L, Stanton PG, Balourdos G, Frydenberg M, de Kretser DM, Robertson DM. Effects of testosterone plus medroxyprogesterone acetate on semen quality, reproductive hormones, and germ cell populations in normal young men. *J Clin Endocrinol Metab* 2002b;87:546-556.

McLachlan RI, Rajpert-De Meyts E, Hoei-Hansen CE, de Kretser DM, Skakkebaek NE. Histological evaluation of the human testis-approaches to optimizing the clinical value of the assessment: mini review. *Hum Reprod* 2007;22:2-16.

McNab FW, Rajsbaum R, Stoye JP, O'Garra A. Tripartite-motif proteins and innate immune regulation. *Curr Opin Immunol* 2011;23:46-56.

Meas T, Deghmoun S, Levy-Marchal C, Bouyer J. Fertility is not altered in young adults born small for gestational age. *Hum Reprod* 2010;25:2354-2359.

Meduri G, Massin N, Guibourdenche J, Bachelot A, Fiori O, Kuttann F, Misrahi M, Touraine P. Serum anti-Müllerian hormone expression in women with premature ovarian failure. *Hum Reprod* 2007;22:117-123.

Meroni G, Diez-Roux G. TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. *Bioessays* 2005;27:1147-1157.

Micci F, Haugom L, Abeler VM, Trope CG, Danielsen HE, Heim S. Consistent numerical chromosome aberrations in thecofibromas of the ovary. *Virchows Arch* 2008;452:269-276.

Milde-Langosch K. The Fos family of transcription factors and their role in tumourigenesis. *Eur J Cancer* 2005;41:2449-2461.

Miller ME, Chatten J. Ovarian changes in ataxia telangiectasia. *Acta Paediatr Scand* 1967;56:559-561.

Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A. Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol secretion before the onset of female puberty in short children. *J Clin Endocrinol Metab* 2000;85:1074-1080.

Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A. Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, and testosterone secretion before the onset of male puberty. *J Clin Endocrinol Metab* 1999;84:29-37.

Mlinar B, Gersak K, Karas N, Zitnik IP, Battelino T, Lukac-Bajalo J. Galactose-1-phosphate uridyl transferase gene mutations in women with premature ovarian failure. *Fertil Steril* 2005;84:253-255.

Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. *Nucleic Acids Res* 1998;26:3453-3459.

Morabia A, Costanza MC. International variability in ages at menarche, first livebirth, and menopause. World Health Organization Collaborative Study of Neoplasia and Steroid Contraceptives. *Am J Epidemiol* 1998;148:1195-1205.

Morohashi K, Honda S, Inomata Y, Handa H, Omura T. A common trans-acting factor, Ad4-binding protein, to the promoters of steroidogenic P-450s. *J Biol Chem* 1992;267:17913-17919.

Morohashi K, Iida H, Nomura M, Hatano O, Honda S, Tsukiyama T, Niwa O, Hara T, Takakusu A, Shibata Y. Functional difference between Ad4BP and ELP, and their distributions in steroidogenic tissues. *Mol Endocrinol* 1994;8:643-653.

Moser HW, Mahmood A, Raymond GV. X-linked adrenoleukodystrophy. *Nat Clin Pract Neurol* 2007;3:140-151.

Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science* 2007;315:201-205.

Murphy BD. Revisiting reproduction: What a difference a gene makes. *Nat Med* 2010;16:527-529.

Myllärniemi S, Koski K, Perheentupa J. Craniofacial and dental study of mulibrey nanism. *Cleft Palate J* 1978;15:369-377.

Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, Krohn KJ, Lalioti MD, Mullis PE, Antonarakis SE, Kawasaki K, Asakawa S, Ito F, Shimizu N. Positional cloning of the APECED gene. *Nat Genet* 1997;17:393-398.

- Nelson LM. Clinical practice. Primary ovarian insufficiency. *N Engl J Med* 2009;360:606-614.
- Nelson LM, Anasti JN, Kimzey LM, Defensor RA, Lipetz KJ, White BJ, Shawker TH, Merino MJ. Development of luteinized graafian follicles in patients with karyotypically normal spontaneous premature ovarian failure. *J Clin Endocrinol Metab* 1994;79:1470-1475.
- Nielsen J, Pelsen B, Sorensen K. Follow-up of 30 Klinefelter males treated with testosterone. *Clin Genet* 1988;33:262-269.
- Niinikoski H, Lagström H, Jokinen E, Siltala M, Rönnemaa T, Viikari J, Raitakari OT, Jula A, Marniemi J, Nantö-Salonen K, Simell O. Impact of repeated dietary counseling between infancy and 14 years of age on dietary intakes and serum lipids and lipoproteins: the STRIP study. *Circulation* 2007;116:1032-1040.
- Noonan JA. Noonan syndrome and related disorders: alterations in growth and puberty. *Rev Endocr Metab Disord* 2006;7:251-255.
- Noonan JA. Noonan syndrome. An update and review for the primary pediatrician. *Clin Pediatr (Phila)* 1994;33:548-555.
- Norio R. Finnish Disease Heritage I: characteristics, causes, background. *Hum Genet* 2003;112:441-456.
- Norio R, Nevanlinna HR, Perheentupa J. Hereditary diseases in Finland; rare flora in rare soul. *Ann Clin Res* 1973;5:109-141.
- Norjavaara E, Ankarberg C, Albertsson-Wikland K. Diurnal rhythm of 17 beta-estradiol secretion throughout pubertal development in healthy girls: evaluation by a sensitive radioimmunoassay. *J Clin Endocrinol Metab* 1996;81:4095-4102.
- Ojajärvi P. The adolescent Finnish child: a longitudinal study of the anthropometry, physical development, and physiological changes during puberty. PhD Thesis. University of Helsinki, Finland, 47-49. 1982.
- Ong AC, Devuyst O. Towards the integration of genetic knowledge into clinical practice. *Nephron Clin Pract* 2011;118:c3-8.
- Orimo A, Inoue S, Minowa O, Tominaga N, Tomioka Y, Sato M, Kuno J, Hiroi H, Shimizu Y, Suzuki M, Noda T, Muramatsu M. Underdeveloped uterus and reduced estrogen responsiveness in mice with disruption of the estrogen-responsive finger protein gene, which is a direct target of estrogen receptor alpha. *Proc Natl Acad Sci U S A* 1999;96:12027-12032.
- Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF-9 and BMP-15 in ovarian function. *Mol Reprod Dev* 2011;78:9-21.
- Ozato K, Shin DM, Chang TH, Morse HC, 3rd. TRIM family proteins and their emerging roles in innate immunity. *Nat Rev Immunol* 2008;8:849-860.
- Palermo GD, Schlegel PN, Sills ES, Veeck LL, Zaninovic N, Menendez S, Rosenwaks Z. Births after intracytoplasmic injection of sperm obtained by testicular extraction from men with nonmosaic Klinefelter's syndrome. *N Engl J Med* 1998;338:588-590.
- Pasquino AM, Passeri F, Pucarelli I, Segni M, Municchi G. Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome. *J Clin Endocrinol Metab* 1997;82:1810-1813.
- Passarge E. Bloom's syndrome: the German experience. *Ann Genet* 1991;34:179-197.
- Paul S. Dysfunction of the ubiquitin-proteasome system in multiple disease conditions: therapeutic approaches. *Bioessays* 2008;30:1172-1184.

- Peltonen L, Jalanko A, Varilo T. Molecular genetics of the Finnish disease heritage. *Hum Mol Genet* 1999;8:1913-1923.
- Perheentupa J. Autoimmune polyendocrinopathy--candidiasis--ectodermal dystrophy (APECED). *Horm Metab Res* 1996;28:353-356.
- Perheentupa J. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab* 2006;91:2843-2850.
- Perheentupa J, Autio S, Leisti S, Raitta C. Mulibrey-nanism: dwarfism with muscle, liver, brain and eye involvement. *Acta Paediatr Scand Suppl* 1970;206:74+.
- Perheentupa J, Autio S, Leisti S, Raitta C, Tuuteri L. Mulibrey nanism, an autosomal recessive syndrome with pericardial constriction. *Lancet* 1973;2:351-355.
- Persani L, Rossetti R, Cacciatori C. Genes involved in human premature ovarian failure. *J Mol Endocrinol* 2010;45:257-279.
- Persani L, Rossetti R, Cacciatori C, Bonomi M. Primary Ovarian Insufficiency: X chromosome defects and autoimmunity. *J Autoimmun* 2009;33:35-41.
- Peterson P, Peltonen L. Autoimmune polyendocrinopathy syndrome type 1 (APS1) and AIRE gene: new views on molecular basis of autoimmunity. *J Autoimmun* 2005;25 Suppl:49-55.
- Pickart CM. Ubiquitin enters the new millennium. *Mol Cell* 2001a;8:499-504.
- Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001b;70:503-533.
- Picton HM, Harris SE, Muruvi W, Chambers EL. The in vitro growth and maturation of follicles. *Reproduction* 2008;136:703-715.
- Pisarska MD, Bae J, Klein C, Hsueh AJ. Forkhead I2 is expressed in the ovary and represses the promoter activity of the steroidogenic acute regulatory gene. *Endocrinology* 2004;145:3424-3433.
- Plant TM. Hypothalamic control of the pituitary-gonadal axis in higher primates: key advances over the last two decades. *J Neuroendocrinol* 2008;20:719-726.
- Price SM, Stanhope R, Garrett C, Preece MA, Trembath RC. The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. *J Med Genet* 1999;36:837-842.
- Purushothaman R, Lazareva O, Oktay K, Ten S. Markers of ovarian reserve in young girls with Turner's syndrome. *Fertil Steril* 2010;94:1557-1559.
- Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *J Histochem Cytochem* 2006;54:385-395.
- Radicioni A, Di Giorgio G, Grugni G, Cuttini M, Losacco V, Anzuini A, Spera S, Marzano C, Lenzi A, Cappa M, Crinò A. Multiple forms of hypogonadism of central, peripheral or combined origin in males with Prader-Willi syndrome. *Clin Endocrinol (Oxf)* 2012;76:72-77.
- Raitta C, Perheentupa J. Mulibrey nanism; an inherited dysmorphic syndrome with characteristic ocular findings. *Acta Ophthalmol Suppl* 1974;123:162-171.
- Rajender S, Singh L, Thangaraj K. Phenotypic heterogeneity of mutations in androgen receptor gene. *Asian J Androl* 2007;9:147-179.
- Rajkovic A, Pangas SA, Ballow D, Suzumori N, Matzuk MM. NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. *Science* 2004;305:1157-1159.

Rajkovic A, Yan MSC, Klysiak M, Matzuk M. Discovery of germ cell-specific transcripts by expressed sequence tag database analysis. *Fertil Steril* 2001;76:550-554.

Rebar RW, Connolly HV. Clinical features of young women with hypergonadotropic amenorrhea. *Fertil Steril* 1990;53:804-810.

Reymond A, Meroni G, Fantozzi A, Merla G, Cairo S, Luzi L, Riganelli D, Zanaria E, Messali S, Cainarca S, Guffanti A, Minucci S, Pelicci PG, Ballabio A. The tripartite motif family identifies cell compartments. *EMBO J* 2001;20:2140-2151.

Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. *J Clin Endocrinol Metab* 1987;65:1231-1237.

Roa BB, Savino CV, Richards CS. Ashkenazi Jewish population frequency of the Bloom syndrome gene 2281 delta 6ins7 mutation. *Genet Test* 1999;3:219-221.

Rodriguez-Wallberg KA, Oktay K. Recent advances in oocyte and ovarian tissue cryopreservation and transplantation. *Best Pract Res Clin Obstet Gynaecol* 2012;26:391-405.

Rome S, Meugnier E, Vidal H. The ubiquitin-proteasome pathway is a new partner for the control of insulin signaling. *Curr Opin Clin Nutr Metab Care* 2004;7:249-254.

Ropers HH. Single gene disorders come into focus--again. *Dialogues Clin Neurosci* 2010;12:95-102.

Roth LM, Czernobilsky B. Perspectives on pure ovarian stromal neoplasms and tumor-like proliferations of the ovarian stroma. *Am J Surg Pathol* 2011;35:e15-33.

Rubio-Gozalbo ME, Gubbels CS, Bakker JA, Menheere PP, Wodzig WK, Land JA. Gonadal function in male and female patients with classic galactosemia. *Hum Reprod Update* 2010;16:177-188.

Sadrzadeh S, Klip WA, Broekmans FJ, Schats R, Willemsen WN, Burger CW, Van Leeuwen FE, Lambalk CB; OMEGA Project group. Birth weight and age at menarche in patients with polycystic ovary syndrome or diminished ovarian reserve, in a retrospective cohort. *Hum Reprod* 2003;18:2225-2230.

Sánchez-Corona J, Morán-Vázquez O, Rivera H, Hernández A, Serrano-Lucas JI, Ocampo-Campos R, Lasso-Avalos L, María-Cantú J. Mulibrey nanism in a Mexican child. *Bol Med Hosp Infant Mex* 1983;40:45-48.

Sarikas A, Xu X, Field LJ, Pan ZQ. The cullin7 E3 ubiquitin ligase: a novel player in growth control. *Cell Cycle* 2008;7:3154-3161.

Sassone-Corsi P. Transcriptional checkpoints determining the fate of male germ cells. *Cell* 1997;88:163-166.

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Patanjali SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NG, Taylor AM, Arlett CF, Miki T, Weissman SM, Lovett M, Collins FS, Shiloh Y. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 1995;268:1749-1753.

Scheffner M, Nuber U, Huibregtse JM. Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. *Nature* 1995;373:81-83.

Schenkman NS, Moul JW, Nicely ER, Maggio MI, Ho CK. Synchronous bilateral testis tumor: mixed germ cell and theca cell tumors. *Urology* 1993;42:593-595.

Schieppati A, Henter JI, Daina E, Aperia A. Why rare diseases are an important medical and social issue. *Lancet* 2008;371:2039-2041.

Schiff JD, Palermo GD, Veeck LL, Goldstein M, Rosenwaks Z, Schlegel PN. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. *J Clin Endocrinol Metab* 2005;90:6263-6267.

Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredsted L, Treier AC, Treier M. The murine winged-helix transcription factor *Foxl2* is required for granulosa cell differentiation and ovary maintenance. *Development* 2004;131:933-942.

Schmidt H, Schwarz HP. Serum concentrations of LH and FSH in the healthy newborn. *Eur J Endocrinol* 2000;143:213-215.

Schrader M, Yoon Y. Mitochondria and peroxisomes: are the 'big brother' and the 'little sister' closer than assumed? *Bioessays* 2007;29:1105-1114.

Schultz N, Hamra FK, Garbers DL. A multitude of genes expressed solely in meiotic or post-meiotic spermatogenic cells offers a myriad of contraceptive targets. *Proc Natl Acad Sci U S A* 2003;100:12201-12206.

Schutgens RB, Ryynanen M, Wanders RJ. Peroxisomal functions in mulibrey nanism. *J Inherit Metab Dis* 1994;17:626.

Schwartz AL, Ciechanover A. Targeting proteins for destruction by the ubiquitin system: implications for human pathobiology. *Annu Rev Pharmacol Toxicol* 2009;49:73-96.

Scott RH, Douglas J, Baskcomb L, Huxter N, Barker K, Hanks S, Craft A, Gerrard M, Kohler JA, Levitt GA, Picton S, Pizer B, Ronghe MD, Williams D; Factors Associated with Childhood Tumours (FACT) Collaboration; Cook JA, Pujol P, Maher ER, Birch JM, Stiller CA, Pritchard-Jones K, Rahman N. Constitutional 11p15 abnormalities, including heritable imprinting center mutations, cause nonsyndromic Wilms tumor. *Nat Genet* 2008;40:1329-1334.

Scott RH, Stiller CA, Walker L, Rahman N. Syndromes and constitutional chromosomal abnormalities associated with Wilms tumour. *J Med Genet* 2006;43:705-715.

Seemanová E, Bartsch O. Mulibrey nanism and Wilms tumor. *Am J Med Genet* 1999;85:76-78.

Sekido R, Lovell-Badge R. Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer. *Nature* 2008;453:930-934.

Sharland M, Burch M, McKenna WM, Paton MA. A clinical study of Noonan syndrome. *Arch Dis Child* 1992;67:178-183.

Shelling AN. Premature ovarian failure. *Reproduction* 2010;140:633-641.

Shelling AN, Burton KA, Chand AL, van Ee CC, France JT, Farquhar CM, Milsom SR, Love DR, Gersak K, Aittomäki K, Winship IM. Inhibin: a candidate gene for premature ovarian failure. *Hum Reprod* 2000;15:2644-2649.

Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* 2003;3:155-168.

Shimasaki S, Moore RK, Otsuka F, Erickson GF. The bone morphogenetic protein system in mammalian reproduction. *Endocr Rev* 2004;25:72-101.

Short KM, Cox TC. Subclassification of the RBCC/TRIM superfamily reveals a novel motif necessary for microtubule binding. *J Biol Chem* 2006;281:8970-8980.

Similä S, Timonen M, Heikkinen E. A case of Mulibrey nanism with associated Wilms' tumor. *Clin Genet* 1980;17:29-30.

Singh RP, Carr DH. The anatomy and histology of XO human embryos and fetuses. *Anat Rec* 1966;155:369-383.

Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001;16:972-978.

Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Pyntikova T, Ali J, Bieri T, Chinwalla A, Delehaunty A, Delehaunty K, Du H, Fewell G, Fulton L, Fulton R, Graves T, Hou SF, Latrielle P, Leonard S, Mardis E, Maupin R, McPherson J, Miner T, Nash W, Nguyen C, Ozersky P, Pepin K, Rock S, Rohlfsing T, Scott K, Schultz B, Strong C, Tin-Wollam A, Yang SP, Waterston RH, Wilson RK, Rozen S, Page DC. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 2003;423:825-837.

Skuse DH, James RS, Bishop DV, Coppin B, Dalton P, Aamodt-Leeper G, Bacarese-Hamilton M, Creswell C, McGurk R, Jacobs PA. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature* 1997;387:705-708.

Starita LM, Parvin JD. The multiple nuclear functions of BRCA1: transcription, ubiquitination and DNA repair. *Curr Opin Cell Biol* 2003;15:345-350.

Steinberg SJ, Dodt G, Raymond GV, Braverman NE, Moser AB, Moser HW. Peroxisome biogenesis disorders. *Biochim Biophys Acta* 2006;1763:1733-1748.

Streblov RC, Dafferner AJ, Nelson M, Fletcher M, West WW, Stevens RK, Gatalica Z, Novak D, Bridge JA. Imbalances of chromosomes 4, 9, and 12 are recurrent in the thecoma-fibroma group of ovarian stromal tumors. *Cancer Genet Cytogenet* 2007;178:135-140.

Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, Yadav-Shah M, Sherman SL. Association of FMR1 repeat size with ovarian dysfunction. *Hum Reprod* 2005;20:402-412.

Sun Y. E3 ubiquitin ligases as cancer targets and biomarkers. *Neoplasia* 2006;8:645-654.

Sutovsky P. Ubiquitin-dependent proteolysis in mammalian spermatogenesis, fertilization, and sperm quality control: killing three birds with one stone. *Microsc Res Tech* 2003;61:88-102.

Suzumori N, Yan C, Matzuk MM, Rajkovic A. Nobox is a homeobox-encoding gene preferentially expressed in primordial and growing oocytes. *Mech Dev* 2002;111:137-141.

Sybert VP, McCauley E. Turner's syndrome. *N Engl J Med* 2004;351:1227-1238.

Takebayashi K, Takakura K, Wang H, Kimura F, Kasahara K, Noda Y. Mutation analysis of the growth differentiation factor-9 and -9B genes in patients with premature ovarian failure and polycystic ovary syndrome. *Fertil Steril* 2000;74:976-979.

Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976;51:170-179.

Tapanainen JS, Aittomäki K, Min J, Vaskivuo T, Huhtaniemi IT. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nat Genet* 1997;15:205-206.

Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, van der Burgt I, Crosby AH, Ion A, Jeffery S, Kalidas K, Patton MA, Kucherlapati RS, Gelb BD. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet* 2001;29:465-468.

Terasawa E, Fernandez DL. Neurobiological mechanisms of the onset of puberty in primates. *Endocr Rev* 2001;22:111-151.

Thoms S, Grønborg S, Gärtner J. Organelle interplay in peroxisomal disorders. *Trends Mol Med* 2009;15:293-302.

- Thorén C. So-called mulibrey nanism with pericardial constriction. *Lancet* 1973;2:731.
- Tong S, Wallace EM, Burger HG. Inhibins and activins: clinical advances in reproductive medicine. *Clin Endocrinol (Oxf)* 2003;58:115-127.
- Tournaye H, Staessen C, Liebaers I, Van Assche E, Devroey P, Bonduelle M, Van Steirteghem A. Testicular sperm recovery in nine 47,XXY Klinefelter patients. *Hum Reprod* 1996;11:1644-1649.
- Treloar AE, Boynton RE, Behn BG, Brown BW. Variation of the human menstrual cycle through reproductive life. *Int J Fertil* 1967;12:77-126.
- Tsai WW, Wang Z, Yiu TT, Akdemir KC, Xia W, Winter S, Tsai CY, Shi X, Schwarzer D, Plunkett W, Aronow B, Gozani O, Fischle W, Hung MC, Patel DJ, Barton MC. TRIM24 links a non-canonical histone signature to breast cancer. *Nature* 2010;468:927-932.
- Tsokos M, Erbersdobler A. Pathology of peliosis. *Forensic Sci Int* 2005;149:25-33.
- Tsuji T, Catusus L, Prat J. Is loss of heterozygosity at 9q22.3 (PTCH gene) and 19p13.3 (STK11 gene) involved in the pathogenesis of ovarian stromal tumors? *Hum Pathol* 2005;36:792-796.
- Tuuteri L, Perheentupa J, Rapola J. The cardiopathy of mulibrey nanism, a new inherited syndrome. *Chest* 1974;65:628-631.
- Ueda M, Kanematsu A, Nishiyama H, Yoshimura K, Watanabe K, Yorifuji T, Mikami Y, Kamoto T, Ogawa O. Testicular thecoma in an 11-year-old boy with nevoid basal-cell carcinoma syndrome (Gorlin syndrome). *J Pediatr Surg* 2010;45:E1-3.
- Urano T, Saito T, Tsukui T, Fujita M, Hosoi T, Muramatsu M, Ouchi Y, Inoue S. Efp targets 14-3-3 sigma for proteolysis and promotes breast tumour growth. *Nature* 2002;417:871-875.
- Vadakkadath Meethal S, Atwood CS. The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain. *Cell Mol Life Sci* 2005;62:257-270.
- Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, Van Steirteghem A, Liebaers I. Cytogenetics of infertile men. *Hum Reprod* 1996;11 Suppl 4:1-24; discussion 25-26.
- van Kasteren YM, Schoemaker J. Premature ovarian failure: a systematic review on therapeutic interventions to restore ovarian function and achieve pregnancy. *Hum Reprod Update* 1999;5:483-492.
- van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, Fauser BJ, Themmen AP, te Velde ER. Serum antimüllerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. *Fertil Steril* 2005;83:979-987.
- van Rooij IA, Tonkelaar I, Broekmans FJ, Looman CW, Scheffer GJ, de Jong FH, Themmen AP, te Velde ER. Anti-müllerian hormone is a promising predictor for the occurrence of the menopausal transition. *Menopause* 2004;11:601-606.
- Vaskivuo TE, Anttonen M, Herva R, Billig H, Dorland M, te Velde ER, Stenbäck F, Heikinheimo M, Tapanainen J. Survival of human ovarian follicles from fetal to adult life: apoptosis, apoptosis-related proteins, and transcription factor GATA-4. *J Clin Endocrinol Metab* 2001;86:3421-3429.
- Veening MA, van Weissenbruch MM, Heine RJ, Delemarre-van de Waal HA. Beta-cell capacity and insulin sensitivity in prepubertal children born small for gestational age: influence of body size during childhood. *Diabetes* 2003;52:1756-1760.
- Verlhac MH, Terret ME, Pintard L. Control of the oocyte-to-embryo transition by the ubiquitin-proteolytic system in mouse and *C. elegans*. *Curr Opin Cell Biol* 2010;22:758-763.

Vernaev V, Staessen C, Verheyen G, Van Steirteghem A, Devroey P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? *Hum Reprod* 2004;19:1135-1139.

Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kieseewetter F, Kohn FM, Schill WB, Farah S, Ramos C, Hartmann M, Hartschuh W, Meschede D, Behre HM, Castel A, Nieschlag E, Weidner W, Grone HJ, Jung A, Engel W, Haidl G. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet* 1996;5:933-943.

Vollman RF. The menstrual cycle. *Major Probl Obstet Gynecol* 1977;7:1-193.

Voorhess ML, Husson GS, Blackman MS. Growth failure with pericardial constriction. The syndrome of mulibrey nanism. *Am J Dis Child* 1976;130:1146-1148.

Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. *J Inherit Metab Dis* 1990;13:802-818.

Wanders RJ, Waterham HR. Biochemistry of mammalian peroxisomes revisited. *Annu Rev Biochem* 2006;75:295-332.

Watkins WJ, Umbers AJ, Woad KJ, Harris SE, Winship IM, Gersak K, Shelling AN. Mutational screening of FOXO3A and FOXO1A in women with premature ovarian failure. *Fertil Steril* 2006;86:1518-1521.

Weidner IS, Møller H, Jensen TK, Skakkebaek NE. Risk factors for cryptorchidism and hypospadias. *J Urol* 1999;161:1606-1609.

Weiss L. Additional evidence of gradual loss of germ cells in the pathogenesis of streak ovaries in Turner's syndrome. *J Med Genet* 1971;8:540-544.

Weissman AM. Themes and variations on ubiquitylation. *Nat Rev Mol Cell Biol* 2001;2:169-178.

Weissman AM, Shabek N, Ciechanover A. The predator becomes the prey: regulating the ubiquitin system by ubiquitylation and degradation. *Nat Rev Mol Cell Biol* 2011;12:605-620.

Weller S, Gould SJ, Valle D. Peroxisome biogenesis disorders. *Annu Rev Genomics Hum Genet* 2003;4:165-211.

Welt CK. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. *Clin Endocrinol (Oxf)* 2008;68:499-509.

Werder EA, Illig R, Torresani T, Zachmann M, Baumann P, Ott F, Prader A. Gonadal function in young adults after surgical treatment of cryptorchidism. *Br Med J* 1976;2:1357-1359.

Wikström AM, Dunkel L. Klinefelter syndrome. *Best Pract Res Clin Endocrinol Metab* 2011;25:239-250.

Winter JS, Faiman C, Hobson WC, Prasad AV, Reyes FI. Pituitary-gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab* 1975;40:545-551.

Woad KJ, Watkins WJ, Prendergast D, Shelling AN. The genetic basis of premature ovarian failure. *Aust N Z J Obstet Gynaecol* 2006;46:242-244.

World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge University Press, Cambridge, UK. 1999.

World Health Organization. WHO laboratory manual for the examination and processing of human semen. WHO Press, Geneva, Switzerland. 2010.

Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA, Carino C, Prasad SV, Skinner SS, Dunbar BS, Dube JL, Celeste AJ, Matzuk MM. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol* 2001;15:854-866.

Young RH, Scully RE. Ovarian sex cord-stromal tumours: recent advances and current status. *Clin Obstet Gynaecol* 1984;11:93-134.

Young RH, Welch WR, Dickersin GR, Scully RE. Ovarian sex cord tumor with annular tubules: review of 74 cases including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. *Cancer* 1982;50:1384-1402.

Zapata JM, Pawlowski K, Haas E, Ware CF, Godzik A, Reed JC. A diverse family of proteins containing tumor necrosis factor receptor-associated factor domains. *J Biol Chem* 2001;276:24242-24252.

Zlotogora J, Sagi M, Cohen T. The blepharophimosis, ptosis, and epicanthus inversus syndrome: delineation of two types. *Am J Hum Genet* 1983;35:1020-1027.

Zolk O, Schenke C, Sarikas A. The ubiquitin-proteasome system: focus on the heart. *Cardiovasc Res* 2006;70:410-421.